

From the Department of Clinical Science,  
Intervention and Technology  
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Karolinska Institutet, Stockholm, Sweden

# **NUTRITIONAL INTAKES OVER TIME AND PERIOPERATIVE NUTRITION IN EXTREMELY PRETERM INFANTS**

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Nutritional intakes over time and perioperative nutrition in extremely preterm infants.

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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**Nihil impossibile est**

***To my husband and our sons.***

# ABSTRACT

**Background** Despite improvements in nutrition practice during the last decades, problems still exist in meeting estimated nutritional needs, especially for extremely preterm (EPT) infants. Today, it is still not known how optimal nutrition and growth will hold in the future. The objectives of this thesis were i) to investigate changes over time regarding nutrition practice in Stockholm and the associations on growth and morbidities such as bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP), and ii) to investigate perioperative nutrition surrounding ductal ligation for patent ductus arteriosus (PDA) of EPT infants born in Sweden.

**Methods** This is a population based study of EPT infants born in Sweden before 27 completed gestational weeks between 2004 and 2011 and who survived >24 h (n=489). Detailed nutritional data retrieved from hospital records including all fluid intakes supplied intravenously and enterally together with anthropometrical data were retrospectively registered in Nutrium, a nutrition calculating software. Comprehensive data on cohort characteristics, neonatal morbidity and mortality were obtained from the Extremely Preterm Infants in Sweden Study (EXPRESS) and the Swedish National Quality register.

**Results** Nutritional intakes in Stockholm have increased continuously over time during 2004 to 2011 and growth improved significantly during later years compared to year-group 2004-2005, (Paper I). The enteral feed advancement and time to full feeds during the year-group 2008-2009 were faster compared to all other years. During the years 2010 to 2011, the proportion of parenteral nutrition was larger and median time to full feeds decreased compared to all other years. Initial growth restriction was reduced by higher energy and protein intake the first week and lower risk of BPD and ROP was associated with higher energy intake the first month, (Paper II). The estimated absolute risk of BPD and ROP decreased when energy intake on days 7 to 27 increased from 110 to 120 and 120 to 130 kcal/kg/d. Reduced risk of BPD was associated with higher energy- and protein intake despite critical illness (>10 days mechanical ventilation), which was not the case in ROP. Perioperative nutrition surrounding ductal ligation for PDA is suboptimal in Sweden, (Paper III). Energy, protein and fat intake were not improved on the third day post-surgery compared to the third day prior surgery. Moreover, protein intake during postneonatal period ( $\geq 28$  days) was significantly lower in infants undergoing ductal ligation compared to those operated between days 7 and 27. Nutritional intakes between NICUs in Sweden differed, particularly regarding intakes of total, enteral and parenteral fluids, (Paper IV). On the day of ductal ligation, nutritional intakes at all NICUs were lower compared to the other perioperative days.

**Conclusions** This thesis highlights the importance of neonatal nutrition. Increased nutritional intake during the first postnatal week and month are associated with reduced initial growth restriction and morbidity. We conclude that there is room for improvement regarding nutritional intakes and growth in general and surrounding perioperative week in particular.

More research is needed to investigate the special nutritional needs of vulnerable, ill EPT infants.

# LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers. The papers will be referred by their Roman numerals (I-IV)

- I. Westin V, Klevebro S, Domellöf M, Vanpée M, Hallberg B, Stoltz Sjöström E. Improved nutrition for extremely preterm infants - A population based observational study.  
Clin Nutr ESPEN. 2018;23:245-251.
- II. Klevebro S, Westin V, Stoltz Sjöström E, Norman M, Domellöf M, Edstedt Bonamy AK, Hallberg B. Early energy and protein intakes are associated with growth and risk of morbidity in extremely preterm infants.  
Clin Nutr. 2018. Pii: S0261-5614(18)30197-3. [Epub ahead of print]
- III. Westin V, Stoltz Sjöström E, Ahlsson F, Domellöf M, Norman M. Perioperative nutrition in extremely preterm infants undergoing surgical treatment for patent ductus arteriosus is suboptimal.  
Acta Paediatr. 2014;103(3):282-288.
- IV. Westin V, Vanpée M, Norman M, Hallberg B, Stoltz Sjöström E. Perioperative nutrition differs between neonatal intensive care units in Sweden.  
Manuscript.



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## LIST OF ABBREVIATIONS

BPD	Bronchopulmonary dysplasia
BMBF	Bovine milk based fortifier
BUN	Blood urea nitrogen
BW	Birth weight
BWSDS	Birth weight standard deviation score
CI	Confidence interval
DHM	Donor human milk
ELBW	Extremely low birth weight
ELGA	Extremely low gestational age
EN	Enteral nutrition
EPT	Extremely preterm
GA	Gestational age
HM	Human milk
HMB	Human milk bank
HMBF	Human milk based fortifier
HMF	Human milk fortifier
MOM	Mothers' own milk
MV	Mechanical ventilation
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
PDA	Patent ductus arteriosus
PN	Parenteral nutrition
RCT	Randomised clinical trial
REE	Resting energy expenditure
ROP	Retinopathy of prematurity
SNQ	Swedish neonatal quality
WHO	World health organisation
WSDS	Weight standard deviation score



# **1 BACKGROUND**

## **1.1 INCIDENCE OF PRETERM BIRTH**

The World Health Organisation's (WHO) definition of preterm birth is birth before 37 completed weeks of gestation (1). About 15 million infants (10 %) around the world are born preterm each year. There are great variations between regions and countries of which the Northern European countries have the lowest rate of premature birth (2). In 2016, the preterm birth rate in Sweden was 5.6 % (3). Preterm birth is divided into three sub-categories: moderate to late preterm (32 to <37 gestational weeks); very preterm (28 to <32 gestational weeks) and extremely preterm or extremely low gestational age (ELGA, <28 gestational weeks) (2). In the Extremely Preterm infants in Sweden Study (EXPRESS), infants born with a gestational age less than 27 completed weeks during a three-year period were included, entailing approximately 0.2 % of all new-borns in Sweden for that time period (4). The aetiology of preterm birth is mainly unknown. However, preterm labour and preterm rupture of membranes often cause preterm birth (5). Preeclampsia, placental haemorrhage, infection, multiple birth and intrauterine growth restriction are also mentioned as reasons for preterm delivery. Other risk factors are e.g., fertility problems, assisted conception, smoking, drug use, extreme under- or overweight.

## **1.2 IMPROVEMENTS IN NEONATAL INTENSIVE CARE**

In Sweden, modern treatment of extremely preterm (EPT) infants based on physiological principles and technological improvements started between the years 1960 and 1970. This, together with increased centralisation of neonatal intensive care units (NICU), resulted in an increase of surviving infants and a decrease of viability limits (6). Improved survival during later years of the last century was at the cost of an increased number of infants with severe disability. A Swedish study published in 1997 showed that none of the infants born in gestational week 22 survived during the years 1990 and 1992 (7). A decade later, results from the EXPRESS cohort including infants born between 2004 and 2007, 51 infants below 23 completed gestational weeks were live-born, of those five infants showed a one-year survival (4). Since infants born at the lower limit of viability require most resources at the NICU and many of them are affected of neurodevelopmental disability, there is concern that remaining resources are not sufficient to meet the follow-up needs of the fragile infant and their families (8). Great improvements in neonatal care have also been reported from England (EPICure study) comparing short term outcomes after extreme preterm birth in 1995 and 2006 (9, 10). As mortality rates and severe sequelae such as cerebral palsy have decreased (11), interest for nutritional management of preterm infants has increased.

### **1.3 NUTRITIONAL SUPPLY**

Between the years 1960 to 1970, nutritional treatment was only introduced when the premature infant was still alive after two or three days' postnatal age (6). Major cumulative nutritional deficits and postnatal growth restriction during the first week of life have been demonstrated in several nutritional studies (12-15). Nutrition should be introduced as soon as possible after birth to prevent deficits that could be detrimental for the most vulnerable infants (16). Neonatal experts have agreed upon consensus guidelines regarding nutritional intakes of EPT infants (16-20).

Before birth, the nutritional supply in utero is mediated through the umbilical cord from the mother's placenta to the foetus. After birth, the nutritional intake is shifting to parenteral nutrition (PN) combined with minimal enteral feeding. Due to the risk for sepsis or PN related liver problems and the need for microbial colonisation of the sterile gut, PN should be substituted as soon as possible by enteral nutrition (EN) (21-24). However, in the preterm infant, limited tolerance to EN may be due to gastrointestinal tract immaturity leading to, impaired motility, digestion and absorption of nutrients (25). It is challenging in EPT infants to prevent nutritional deficits and to reach optimal growth due to the infants' limited stores of nutrients at birth and the difficulties to supply optimal amounts of both energy and nutrients after birth (26).

#### **1.3.1 Parenteral nutrition**

PN is usually administrated through central venous catheters and is the main supply of nutrition during the first postnatal week. In a study of Skouroliahou et al, two types of total PN prescription methods in preterm infants were compared (27). The use of protocols prescribed by individual physicians resulted in less adequate provision of nutrients, weight gain and worse blood count profile compared with standardised protocols. The prescription of different physicians can be easily affected due to subjective factors such as the current clinical status of the infants and lead to less aggressive prescriptions.

Figure 1 shows an example of individualised PN composition.



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**Figure 1.** Individual composition of parenteral nutrition according to a protocol prescribed by individual physicians including protein, carbohydrates and micronutrients (bag) as well as fat and vitamins (syringe).

Optimal nutritional intakes during the first week of life are difficult to reach and the risk for increased cumulative nutritional deficits is large (12-15). In a recent Belgian study, the nutritional protocol was adapted to current guidelines for EPT infants (16-18). The infants received 40 kcal/kg/d and 2.5 g protein/kg/d the first postnatal day and 120-130 kcal/kg/d with 3.8-4.4 g/kg/d of protein by the end of the first postnatal week (28). As a result, the optimised nutritional intakes during the first week lead to improved growth and a reduction in postnatal cumulative nutritional deficits. Furthermore, the use of PN with an optimised, standardised, ready-to-use solution contributed to improving the early nutritional supply.

In a randomised clinical trial (RCT), Morgan et al showed that premature infants who received a standardised, concentrated PN solution during the first 28 days of life ameliorated their head growth (29). Miller et al. showed improved nutritional intakes and growth by implementing a nutrient based-protocol warranting sufficient energy and protein intakes during the transition phase of nutrition when PN is weaned with advancing enteral feeds (30). In a randomised double-blind controlled trial by Uthaya et al, premature infants were divided into four groups each receiving one of four PN solutions that differed in lipid composition and amount of amino acids provided during the first day of life (31). Expected differences between the four groups regarding amounts of intra-hepatocellular lipid and lean body mass were not demonstrated. The amount of 3.6 g amino acids intake on day one did not benefit body composition nor growth to 36 postnatal weeks and may be harmful.

### 1.3.2 Enteral nutrition

Human Milk (HM) delivers optimal nutrients for infant growth, enables maturation of the intestine, microbial colonisation and intestinal motility (21, 23). Important outcomes such as risk for necrotising enterocolitis (NEC) and late onset sepsis may be associated with the timing of introduction and the rate of advancement of enteral HM feeds (32, 33). Colostrum is breast milk produced during the first postnatal days after birth, Figure 2. During six postnatal weeks after birth the protein content in breast milk decreases to be less than half of the colostrum content (34).

Giving small volumes (12-24 ml/kg/d) of HM without advancing the feed volumes during the first five to seven days after birth is defined as early trophic feeding (35). The definitions for early and delayed introduction of HM feeds are up to four days and later than five to seven days of postnatal life, respectively (36).

Furthermore, slow and fast advancement as daily increments of HM feeds are defined as 15-20 ml/kg/d and 30-35 ml/kg/d, respectively (37).



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**Figure 2.** Colostrum in syringe.

Delayed introduction and slow advancements of enteral feeds prolong the need for PN and also the time to regain birth weight. However, the long-term clinical importance of these effects is still unclear. Compared to premature formula, HM is associated with shorter hospital stay (38) and lower incidence of re-hospitalisation (39). Mother's own milk (MOM) or donor breast milk (DHM) is always the first-hand choice for EN in Swedish NICUs (40). The health benefits of HM have been increasingly recognised (41). MOM is given to the preterm infants as long as the milk production last. When MOM production is suboptimal, a complement with DHM is given, until the infant reach a postnatal age of at least 32-35 weeks. Premature formula can be used at the earliest at 32 weeks of postnatal age but only when supply of MOM is insufficient or terminated (42). Macronutrients' content in MOM and DHM are analysed on a regular base (43). Mid-infrared spectrophotometry has been used to determine macronutrient content in dairy products for many years. When calibrated for measurement of macronutrients in HM, the mid-infrared spectrophotometry technique facilitates individual fortification of HM (44, 45). As macronutrients' content in HM changes over time, especially regarding protein (46), regular macronutrient analyses of MOM are needed, preferably at least weekly during the first postpartum weeks (47). Early MOM, expressed during the first 28 postnatal days usually contains more protein especially during the first two postnatal weeks compared to mature MOM (>28 days), Figure 3, in contrast to



DHM's lower protein content because of its earliest expression after the first two postnatal weeks (48).



**A**

**B**

**C**

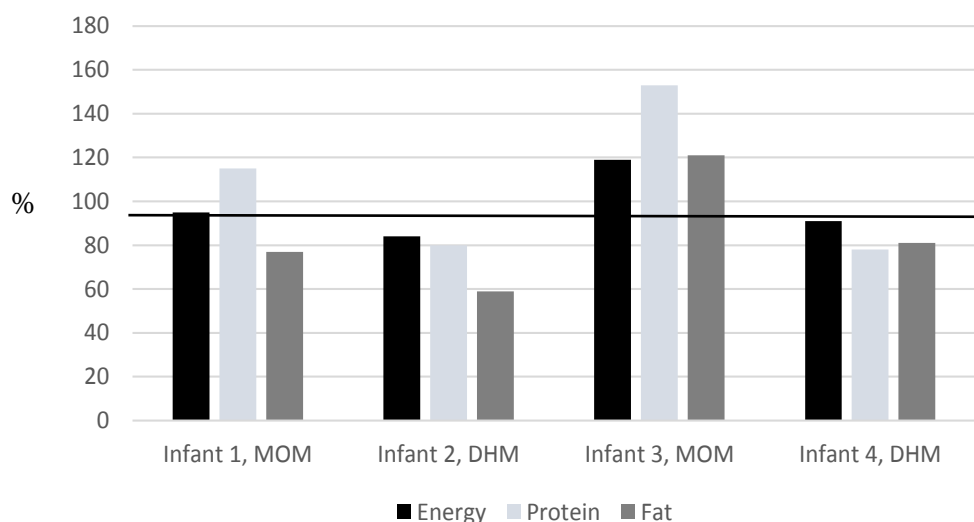
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**Figure 3.** Differences in colour and nutrient content of mothers own breastmilk.

<b>Nutrient content g/100 ml</b>	<b>Protein</b>	<b>Fat</b>	<b>Carbohydrate</b>
MOM A	1.0	2.2	7.1
MOM B	1.4	4.0	7.2
MOM C	1.0	3.2	7.4

In Sweden, there are 28 human milk banks (49), which is the highest number per capita in the world. To be able to follow specific safety guidelines DHM should be provided from established human milk banks (50). Some biological components in DHM are reduced by the storage and processing and may diminish the health benefits. The focus of future research should be on the improvement of milk processing in human milk banks, particularly of heat treatment and optimisation of HM fortification. Furthermore, research is needed to investigate the potential clinical benefits of processed and fortified DHM.

During the last decades, most research regarding EN is based on fixed amounts of fortifications or standardised fortification, in HM that has not been analysed, assuming an average content of macronutrients and energy in HM (51-54). Since there is an inter- and intra-individual variation of the composition in HM, target dosage by individualised fortification rather than standard fortification should be applied to avoid under- or over nutrition of the infant, Figure 4 (46, 47, 55-62).



**Figure 4.** Bar chart demonstrating four hypothetical fluid restricted infants (160 ml/kg/d) on standard fortified mothers' own milk and donor human milk (0.8 g protein per 100 ml). The black line denotes estimated nutritional needs for full enteral feeds, including 135 kcal/kg/d, 4 g protein/kg/d and 7 g fat/kg/d (47E %), according to The Swedish Board of Health and Welfare (20).

Another type of individualised fortification is called adjustable fortification using blood urea nitrogen values twice a week to assess the amount of HM fortifications needed with regard to protein (63). This can delay increases in protein intake which allows malnutrition to some extent. Moreover, blood urea nitrogen does not give any information regarding energy and fat intakes.

## 1.4 ESTIMATED NUTRITIONAL REQUIREMENTS

American and European guidelines published during 2005 and 2010 (16-19), differ slightly compared to contemporary guidelines (64, 65). Based on previous guidelines, in 2014, The Swedish National Board of Health and Welfare published nutritional guidelines for extremely preterm infants born before 28 completed weeks of gestation, Table 1 (20).

**Table 1.** Nutritional guidelines for extremely preterm infants according to The Swedish National Board of Health and Welfare.

	Day 1	Day 4	Enteral nutrition full dose	Total parenteral nutrition full dose	Unit (/kg/d)
<b>Energy</b>	50-60	100-120	115-135	90-115	Kcal
<b>Protein</b>	2.0-2.4	3.5-4.5	4.0-4.5	3.5-4.0	g
<b>Fat</b>	1.0-1.5	4.0-6.0	5.0-8.0	3.0-(4.0)	g
<b>Carbohydrate</b>	8.0-11.0	10.0-15.0	9.0-15.0	11.4-14.4	g
<b>Fluid</b>	80-120	130-160	135-200	135-180	ml

### 1.4.1 Energy

In infancy, resting energy expenditure (REE) is the appropriate method used to measure the amount of energy needed to maintain the vital processes of the body in stable infants (65). REE is not higher than 10 % compared to the basal metabolic rate and can be measured in a thermo-neutral environment, most suitable before feeding or after a fasting period. REE is decreased in hypothermia and increased in conditions such as e.g., fever, inflammation and chronic diseases. To date, there are no Schofield's equations for calculating REE for premature infants.

Energy supply should cover basal metabolic rate and growth and also compensate for negative energy balance. Nevertheless, according to a recent study, PN practices are frequently not compliant with current guidelines, especially during the first postnatal days of life (66). Swedish guidelines suggest the intake of 50-60 kcal/kg/d on the first day of life and intake of 90-115 kcal/kg/d from full PN, Table 1 (20). In contrast, recent ESPGHAN guidelines recommend 45-55 kcal/kg/d on the first day of life regarding premature neonates and 90-120 kcal/kg/d from full PN including extremely low birth weight infants (65). Transition time from PN to full EN in extremely preterm infants can take up to several weeks (67). Swedish guidelines suggest the intake of 115-135 kcal/kg/d from full EN, Table 1 (20). According to these guidelines, an energy intake of 100-120 kcal/kg/d, consisting of 50 % EN and PN is proposed already on the fourth day of life.

To calculate energy in kilocalories (kcal), energy factors by Atwater's are used (68). The energy factor is 4 kcal per gram and 9 kcal per gram for protein, carbohydrates and fat, respectively. However, the energy available from macronutrients differs between PN and EN. Because of higher splanchnic tissue metabolism and stool losses, energy requirements are generally 10-20 % higher with enteral feeds are compared to PN (69-72).

### 1.4.2 Protein

Because of the need of invasive methods, little is known about the protein requirements or metabolism in the human foetus (73). The body composition analysis of still born preterm infants constitutes the basis for the current available standards for intrauterine protein accretion, suggesting that the foetus during the third trimester grows at about 15 g/kg/d. Furthermore, the protein accretion is approximately 2 g/kg/d and decreases slowly towards term age (26). Net protein accretion by protein synthesis exceeding the protein break down, is necessary to increase the lean body mass. In the preterm infant, the protein synthesis exceeds the protein intake leading to protein turnover since proteins are continuously broken down in the body and resynthesized (73). According to the nitrogen balance method approximately 1.5 g/kg/d is initially needed for a neutral net protein accretion rate (18).

Swedish guidelines suggest a protein intake of 2.0-2.4 g/kg/d on the first day of life and a protein intake of 3.5-4.0 g/kg/d from total PN (20). Recommendations regarding protein

intakes are different depending on the new-born infant's birth weight. According to the Swedish guidelines, the protein intakes should be 4.0-4.5 g/kg/d for EPT infants weighing 1000 gram or less and 3.5-4.0 g/kg/d for those weighing between 1000 gram to 1800 gram (20). On the fourth postnatal day if fluid intakes, consisting of 50 % EN are reached, Swedish guidelines stipulate protein intake of 3.5-4.5 g/kg/d (the higher amount for infants < 1000 grams). During the last decades, several studies have highlighted the importance of sufficient early amino acid supply for a positive protein accretion rate (74-78). To facilitate maximal protein accretion, energy intake of at least 120 kcal/kg/d is the aim of most practitioners (79, 80). Since it is likely that protein and other nutrients are limiting factors regarding growth, protein to energy (PE) ratios were calculated, based on grams of protein provided per 100 kcal. The ideal PE ratio is yet not known (65) and different PE ratios such as 3.2 to 4.1 g protein per 100 kcal (18) and 3.1 to 3.3 g/100 kcal (81) are suggested in recent literature.

### **1.4.3 Fat**

Fat contains about twice as much calories per gram compared to other macronutrients and is therefore an effective source of energy. Failure to provide fat as soon as possible after birth may cause suboptimal calorie intake and proteolysis (16). The intrauterine provision of fat is low (82) and the EPT infant have low fat stores at birth. Furthermore, it is difficult to meet estimated nutritional fat needs because of reduced digestion and absorption from the intestines (83) and reduced enzyme activity in DHM due to pasteurisation (84). Recent studies have demonstrated positive effects of early parenteral fat on nitrogen balance and thereby improved conditions for anabolism (77, 85). Vlaardingerbroek et al demonstrated higher nitrogen balance on day 2 and lower plasma urea, when a high dose parenteral lipids of 2.0-3.0 g/kg/d were combined with 2.4 g amino acids from birth (77). Moreover, higher intakes of fat (3.0 g lipids/kg/d) and amino acids (3.5 g/kg/d) already on the first postnatal day were well tolerated (85). However, fat malabsorption is common among preterm infants because of an immature exocrine pancreatic function at birth (83). A Swedish study of 29 preterm infants born between 32 and 37 postnatal weeks, demonstrated at full term age a higher percentage of body fat when compared with new born infants matched for body weight and GA (86).

Furthermore, fat also supplies essential fatty acids, which are needed for the structural composition and the myelination of the brain and retina (87). The long chain polyunsaturated fatty-acid (LC-PUFA) n-6 is potentially pro-inflammatory and the main source of lipids in several PN solutions. A reduction PN solutions containing soybean oil (n-6 fatty acids) may be indicated in premature infants with lung function problems since n-6 influence the pulmonary vasculature (88, 89). Alternative emulsions containing oil mixtures, rich in olive oil (n-9) are preferred since they reduce the risk of sepsis and promote a more favourable LC-PUFA profile. A recent RCT in extremely preterm infants on PN, supplemented with SMOFlipid®, containing 15 % fish oil (n-3 fatty acids), demonstrated neither reduction of morbidity nor effect on growth (90). Furthermore, docosahexaenoic acid (DHA) was

marginally increased and arachidonic acid (ARA) to DHA ratio was decreased. More studies regarding DHA and ARA are needed since EPT infants accumulate a large deficit of these essential fatty acids.

According to Swedish guidelines the fat intake from PN and EN is 3.0-4.0 g/kg/d and 4.0-8.0 g/kg/d, respectively, (20). Already on the fourth postnatal day the recommended fat intake of 4.0-6.0 g/kg/d and fluid intakes, consisting of 50 % EN should be reached in EPT infants' nutrition. Swedish guidelines stipulate enteral fat intake of between 40 and 55 Energy %, corresponding to approximately 8.0 g fat/kg/d or even more.

#### **1.4.4 Carbohydrates**

Carbohydrates and specifically glucose are the most important sources for many metabolic processes and particularly for the brain and the heart in the preterm infant (17). A minimum glucose intake of 4.0 mg/min/kg, approximately 7.0 g/kg/d is needed to meet the glucose requirements of the brain (91). The novo synthesis of fatty acids and a number of non-essential amino acids is provided by carbon in glucose (64). During the first days of life, most preterm infants produce endogenous glucose corresponding to 4.0-5.0 mg/min/kg from both glycogenolysis and gluconeogenesis (92, 93). There is a risk for hyperglycaemia during the first postnatal week because of sustained endogenous glucose production despite supply of parenteral glucose. A common way to treat infants' hyperglycaemia is to reduce glucose infusion. Insulin treatment favours nutritional intake but is seldom used in Sweden (94). Swedish guidelines recommend a parenteral carbohydrate intake of 13.0-17.0 g/kg/d (20).

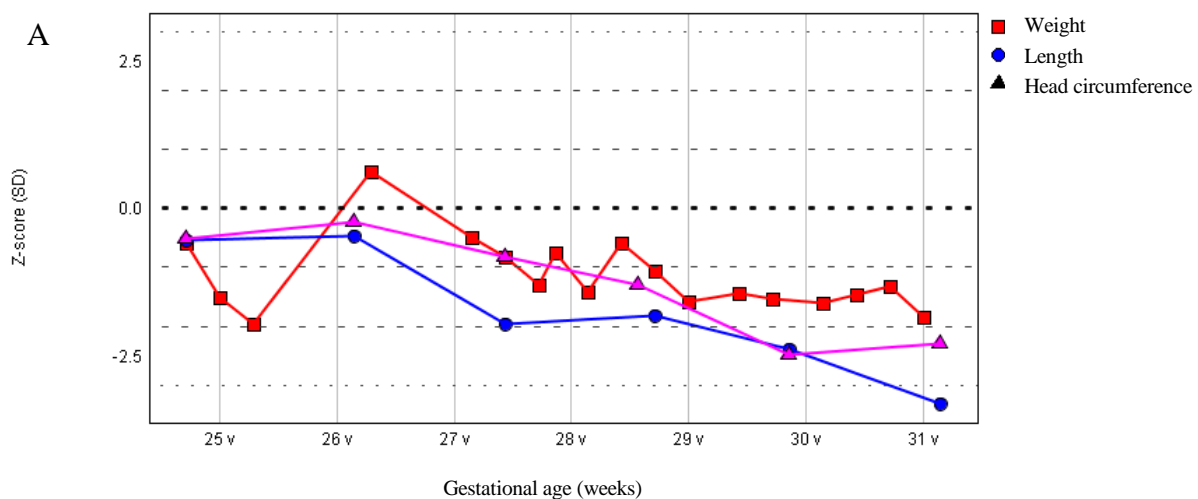
Lactose is a disaccharide composed by galactose and glucose the main carbohydrate in HM, and the enzyme lactase in the small intestine is necessary for reduction and absorption (25). In preterm infants, lactase activity is 30 % lower, compared to term infants, causing lactose malabsorption. Malabsorption of lactose is detrimental for preterm infants' health, leading to feeding intolerance, delayed attainment of full enteral feeds, and poor weight gain (95-97). Shulman et al found that higher lactose absorption in preterm infants was more related to improved lactase activity than to mucosal growth (98). Moreover, a relation was showed between enteral feeds and mucosal growth. Swedish guidelines recommend an enteral carbohydrate intake of 9.0-15.0 g/kg/d (20). On the fourth postnatal day if EN reaches 50 %, the recommended carbohydrate intake is 11.0-16.0 g/kg/d.

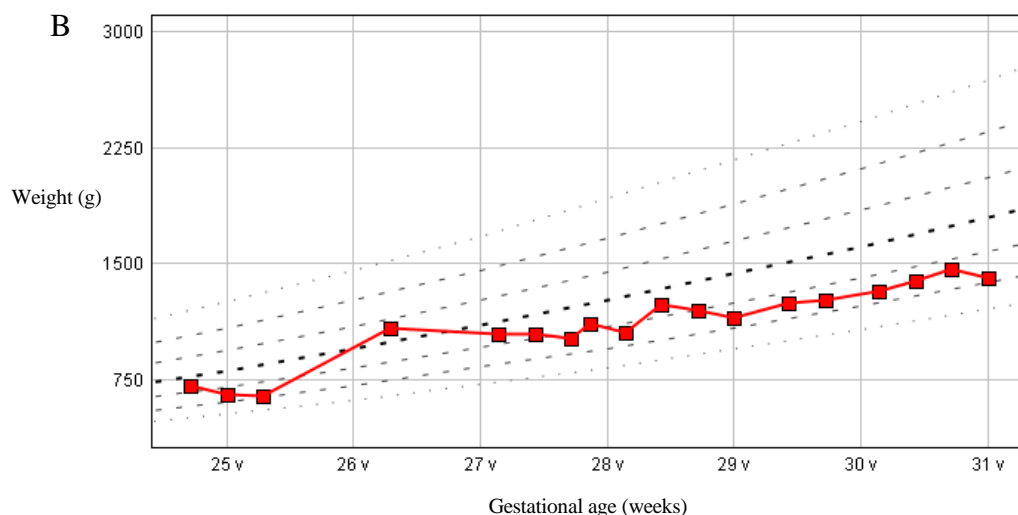
### **1.5 POSTNATAL GROWTH**

Despite improved neonatal care and enhanced neonatal nutrition during the last decades, postnatal growth in premature infants is still suboptimal (12-15, 99). Since optimal growth standards have not been defined, the optimal growth of preterm infants is considered to be equivalent to intrauterine growth patterns of the foetus (18, 19, 100).

### 1.5.1 Growth charts

In the beginning of the 1990s, Gardosi and colleagues introduced a standard, longitudinal ultrasound-derived growth curve for intrauterine weight gain, customised for each individual pregnancy, taking the mother's characteristics and birth weights from previous pregnancies into consideration (101). Growth curves based on data from four Scandinavian NICUs regarding ultrasonically estimated foetal weights in uncomplicated pregnancies was performed in 1996 by Marsal et al (102). Later on, Niklasson and Albertsson-Wikland introduced continuous and smoothed, gender specific growth reference values from 24<sup>th</sup> week of gestation to 24 months of age, based on a Swedish national birth cohort between 1990 and 1999 (103). These curves, also based on estimated normal growth in utero, are suitable especially for the follow-up of preterm infants because of the visual presentation of age corrected growth curves. However, most published postnatal growth trajectories for preterm infants have been established by studying NICU cohorts, inevitably consisting of a mix of sick and healthier infants (104-108). Figure 5 demonstrates growth curves in Nutrium, based on Niklasson and Albertsson-Wikland's gender specific growth reference values. Apart from a calculating tool, Nutrium is also used to visualise premature infants' growth in many Swedish NICUs.





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**Figure 5.** Growth charts in Nutrium according to Niklasson and Albertsson-Wikland, demonstrating growth during a seven weeks' period of an extremely premature infant born before 25 weeks of gestational age and a weight of 711 grams, visualised as standard deviation scores (A) and weight growth curve in grams (B). The black dotted lines denote estimated normal growth in utero.

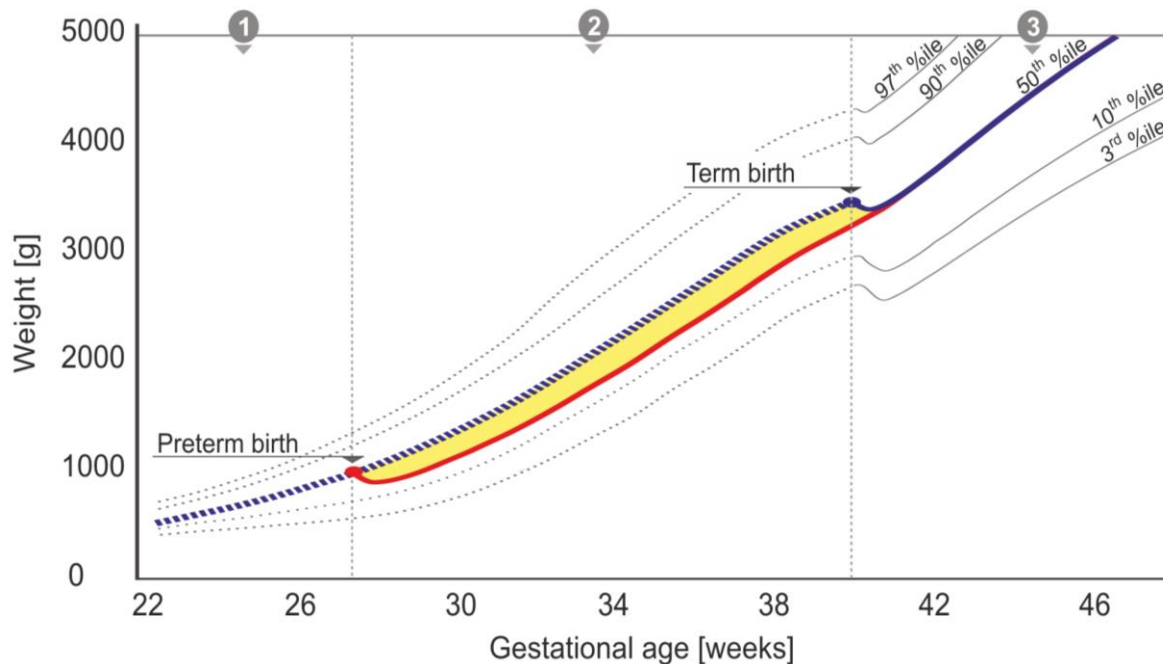
In 2004, the WHO published a multicentre growth reference study considered as preferred standard for postnatal growth (109). Those postnatal growth charts begin at term age and should not be used to assess preterm postnatal growth before 36 completed weeks of gestation. The Paediatric societies in Europe (18) and North America (19) have recommended that the aim of nutritional guidelines is to generate optimal postnatal growth matching the in utero growth and mimicking the foetal body composition until full term. In 2010, Olsen et al published new intrauterine growth curves based on contemporary, large, ethnically diverse, American data (110).

In a systematic review and meta-analysis, Fenton et al demonstrated the revised preterm growth chart, in accordance with the WHO standard at 50 weeks' postnatal age (111). These growth patterns may support an improved transition of preterm infant growth monitoring of the WHO growth trajectories and show a gender specification regarding premature infants. In 2016, Rochow et al provided target trajectories for extrauterine growth after completed postnatal adaption, based on healthy preterm infants born between 25 and 34 weeks of gestation with little or no clinical interventions required (112).

## 1.6 NUTRITIONAL INTAKES AND POSTNATAL GROWTH

One of the critical limitations of using preterm infants as a growth reference is the changing growth pattern caused by the improvement of preterm nutrition and medical care (106). Rochow et al demonstrated a shift in the infants' postnatal trajectory by -0.8 standard deviation scores (SDS) caused by an irreversible contraction of extracellular water space

during the first postnatal days (113-118) and by the abrupt lapse of placental nutritional supply, Figure 6 (112). The sudden lack of placental nutritional supply after birth combined with suboptimal nutritional support such as delayed postnatal enteral feeding advancement, prolonged use of suboptimal PN and repeated incidences of feeding intolerance increases the risk for nutritional deficit in the premature infant. However, this observed postnatal offset might be temporary and disappear with optimal nutrition, normal environmental conditions and under normal physiology.



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**Figure 6.** Postnatal offset of growth charts illustrated by the course of one hypothetical infant. Dotted (blue) and solid (red, blue) lines represent intrauterine and postnatal growth curves, respectively, and solid (yellow) space represents the deviation from intrauterine growth curve. The hypothetical infant's intrauterine growth is along the 50<sup>th</sup> percentile (1) but deviates after birth from the intra uterine growth curve due to redistribution of fluid (2) according to Fenton et al (106). The infant's continued growth is on a curve parallel to its peers still in utero, but after 42 weeks, back to the 50<sup>th</sup> percentile (3) according to WHO's postnatal charts (109). In recent guidelines of Joosten et al, a weight gain of 17-20 g/kg/d was recommended for very low birth weight infants to prevent continued weight drop across the centiles after the postnatal nadir of weight loss (65).

There is a lack of standardised approach to report growth and nutritional intake data in neonatal literature (119). An important factor contributing to the lack of progress in this field is the variability in reporting which makes it difficult to compare studies and to rely on meta-analysis. To facilitate the use of nutrition and growth data, Cormack et al proposes the Checklist-Standardized Reporting Of Neonatal Nutrition and Growth (StRONNG) (119). Because of improved survival rates of very preterm infants, the focus of neonatal care has shifted onto improving postnatal growth and nutrition to achieve growth rates to optimise later health outcome (120). Recent studies show that there is a relationship between given nutrition and growth during the neonatal period (29, 52, 99). Nutritional outcomes in these



studies are based on detailed information on nutritional intakes regarding energy and macronutrients in PN and EN, respectively.

A prospective cohort study, including preterm infants showed a lower growth rate compared to the intrauterine growth curve despite achieving energy and protein intakes close to recent nutritional recommendations (121). Another study demonstrated a reduction in postnatal cumulative nutritional deficit and an improvement of growth in preterm infants born before 31 weeks of gestation infants, when optimising the nutritional practice in the NICU (28). In conclusion, it is difficult to achieve optimal nutrition to generate optimal growth, and more evidenced based research in this field are warranted.

## **1.7 NEONATAL MORBIDITY AND NUTRITION**

The third trimester is very important regarding organ development, not at least regarding the brain (122). Conditions for normal growth and development are changed by preterm birth. Preterm infants, particularly the smallest ones, suffer from several different neonatal problems due to immaturity at birth, short- and long-term morbidity occur at a higher content with decreasing GA (123-126). Lower BW compared to GA is in itself a risk factor for morbidity (127). Clinical risk index for babies (CRIB) is used to establish severity of neonatal morbidity (128). In this neonatal score system, in which high CRIB score is equal to high risk, and information during the first twelve hours of life regarding e.g., BW, GA, presence of congenital malformations, maximum and minimum expired oxygen are included. Increased risk of cerebrovascular complications has been associated with a high CRIB score (125). The Apgar-score was developed in the beginning of the 1950s, this is a well-used system to determine infants' condition at birth (129). Characteristics such as heart rate, respiratory effort, muscle tone, irritability and colour are scored at one, five and ten minutes after birth. A score of seven or higher, based on characteristics assigned value of maximum two, indicates good or excellent condition of the new-born infant.

### **1.7.1 Bronchopulmonary dysplasia**

Premature infants born between 23 to 30 weeks of gestation are at risk for bronchopulmonary dysplasia (BPD), a respiratory disorder that usually occurs after respiratory distress syndrome (130). Some infants suffering from severe lung disease require supplemental oxygen during several weeks and sometimes months after birth (131). The most accepted clinical definition for severe BPD is defined as the need for at least 30 % oxygen at 36 weeks of GA (132). Several studies showed that suboptimal growth increases the frequency of BPD in preterm infants (127, 133, 134). Approximately 50 to 80 % of EPT infants are affected of BPD a disease originating from impaired development of the lungs (123, 125). Incidence and severity of BPD has been associated with suboptimal growth among ELGA infants (135-137). One RCT observed a reduction in occurrence of BPD when preterm infants born <30

weeks of gestation were fed with fortified DHM compare to premature formula (138). To date, this RCT is the only one performed with fortified DHM.

### **1.7.2 Retinopathy of prematurity**

Approximately 50 to 80 % of EPT infants are affected by retinopathy of prematurity (ROP), a disease originating from impaired development of retina (123, 125). Infants born extremely preterm are at high risk to develop ROP, leading to high risk of visual impairment and of blindness. Well known risk factors for ROP development are prematurity itself, low birth weight, oxygen exposure (139) and poor weight gain during the first weeks of life (140, 141). Disturbed vascular development of the retina is the cause of ROP. There are five severity stages in ROP detected by eye examination (142) and stage 3-5 is considered severe ROP. Incidence and severity of ROP has been associated with suboptimal growth among ELGA infants (135, 137, 143).

In a randomised clinical trial (RCT), the intervention group that had higher initial infusion rate of parenteral lipids, leading to higher energy intake during the first postnatal week, had a lower rate of ROP (144). Energy intake at the lowest quartile the first 28 postnatal days was associated with increased risk of severe ROP, according to a study of Vander Veen et al (145). In the EXPRESS cohort, the association between nutrition and ROP development was also investigated, demonstrating that a 10 kcal/kg/d increase in energy intake the first 28 postnatal days, adjusted for morbidity, was associated with a decreased risk of severe ROP (146). Contrary to higher intakes of energy, fat and carbohydrates, protein was not associated to a lower risk of severe ROP.

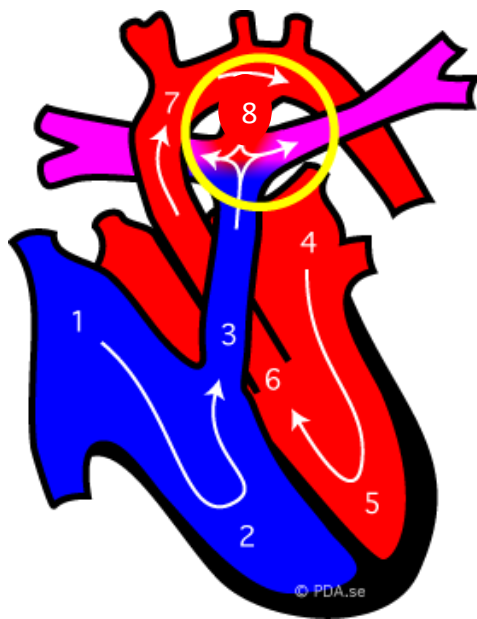
### **1.7.3 Patent ductus arteriosus**

Patent ductus arteriosus (PDA) is a common neonatal morbidity. In the EXPRESS cohort 61 % of infants required treatment for PDA (125). In utero, a blood vessel called ductus arteriosus connects the main pulmonary artery to the descending aorta, Figure 7. In term infants the ductus closes within the first 24 to 48 hours after birth (147), while spontaneous closure in preterm infants often is delayed. Furthermore, the closure rate declines in preterm infants born at low gestational ages (148).

Shunting of blood from the aorta to the pulmonary artery (left to right shunt) occurs through the PDA when flow resistance of the lung cycle decreases (20). With a large PDA, symptoms such as increases in respiratory rate and heart rate arise as well as increases in oxygen dependency and respiratory support. Other complications due to a large PDA might be heart failure, apnoea, fall in blood pressure and kidney failure. Moreover, a large PDA affects postprandial perfusion of the superior mesenteric artery and is associated with impaired feeding intolerance (149) causing e.g., extended time to full enteral feeds (150).

When PDA causes hemodynamic effects, the first line of treatment is with pharmaceuticals (Ibuprofen or Indomethacin) and the second line of treatment is with surgical ligation (151).

Today, there is no consensus about optimal postnatal age for pharmaceuticals or ductal ligation for PDA (152, 153). PDA in preterm infants is associated with increased risk for other morbidities, such as BPD, intraventricular haemorrhage and NEC (154). However, it is unclear if this association is with the hemodynamic effects of the left-to-right shunt; the pharmacological/surgical treatment, or the immaturity (151). Physiological preoperative stabilisation is necessary including the withholding of enteral feeds during at least 6 hours prior ductal ligation (155).



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**Figure 7.** Blue, including numbers one, two and three denotes right atrium, right ventricle and pulmonary artery, respectively. Red, including numbers four, five, six, seven and eight denotes left atrium, left ventricle, aorta, aorta arches and ductus, respectively.

A Cochrane review showed a decreased risk of PDA with restricted fluid intake during the first days after birth in preterm infants (156). Fluid restriction makes it much more difficult to achieve adequate nutritional supply and the need of more concentrated nutritional products such as parenteral solutions might therefore be necessary to reach nutritional goals for ELGA infants.

#### 1.7.4 Necrotising enterocolitis

Necrotising enterocolitis (NEC) is an inflammatory bowel necrosis and is the major cause of morbidity and mortality in EPT infants (157). Any segments of the intestine may be involved

but the terminal ileum and the proximal colon are at highest risk for being affected. The classification system including three different stages was presented by Bell et al in 1978 and modified by Kliegman and Walsh nine years later, is used for determining the severity of NEC (158, 159). The incidence of NEC in Sweden and USA is 5.8 % among infants with GA <27 weeks (125) and 11 % among infants with GA <29 weeks (123), respectively.

Several studies from the last decades found that HM feedings in the NICU are associated with decreased morbidity and mortality, regarding NEC (51, 138, 160-163). Three systematic reviews (164-166), comparing DHM versus formula suggest that DHM has a protective effect against NEC in premature infants. Today, there is lack of evidence regarding the effect of individualised fortified HM on tolerance and incidence of NEC. Current evidence from the Cochrane reviews suggest that the introduction of HM before the postnatal age of four days and the advancement of the rate of feed volumes at more than 24 ml/kg/d do not increase the risk of NEC in VLBW infants (167). A recent American study found no differences in NEC rates, comparing infants fed exclusively preterm formula with infants receiving mixed diet containing less than 98 % of HM (168). In this study, HM was fortified (standard fortification), but no information about type or quantity of the bovine milk based fortifiers (BMBF) was supplied. Other studies showed a reduction in the probability of requiring PN (169) and lower rates of NEC (170) when HM was fortified with human milk based fortifiers (HMBF) instead of BMBF. One group received HMBF, while the other group received BMBF and premature formula. A recent RCT could not demonstrate differences in feeding tolerance among infants born below 1250 grams who were fed with MOM and or DHM fortified with either HMBF or BMBF (171).

Osmolality is the number of osmoles of a solute in a kilogram of solvent, reflecting the solute's concentration, expressed in mOsm/kg. Osmolality of feeds vary, the lowest values, 300 mOsm/kg are found in HM and the highest values, just more than 400 mOsm/kg in fully fortified HM (172). Moreover, mineral and vitamin supplements added to small volumes of milk should be avoided if possible, since osmolality can be highly increase. High osmolality of enteral feeds delays gastric emptying and has been linked to the pathogenesis of NEC (173, 174). However, Pearson et al did not found evidence for a connection between osmolality of feeds and the development of NEC (172).

### **1.7.5 Other morbidities**

Infections in preterm infants occur frequently and sepsis is a common serious condition. In a recent Cochrane review, the association between nosocomial infections and the use of percutaneous central venous catheter and peripheral cannulae was assessed (175). The prevalence of catheter-related sepsis in the NICUs reached 40 %, depending on the population studied (176-178). A vicious cycle emerges, starting with feeding intolerance, slow advancements of EN and outstretched need of vascular access for PN subsequently

leading to sepsis. Growth failure has been associated with sepsis, but not GA or BW in a Swedish population based study (125).

Intraventricular haemorrhage (IVH) is a brain injury that evolves during the first week of life, particularly, the first three postnatal days (179). IVH are classified into four different grades of which grade three to four are rated as severe IVH (180). IVH grade 3 and grade 4 are IVH with ventricular dilatation possibly leading to hydrocephalus and intraventricular bleeding leading to infarction in the brain parenchyme, respectively.

## **1.8 LONG-TERM OUTCOME**

Long-term outcomes such as suboptimal neurodevelopment and cardiovascular risks factors are common present in preterm infants. Extremely premature infants are at increased risk of neurodevelopmental disabilities, including cerebral palsy, cognitive disability, and disabilities caused by vision and hearing impairments (181). There are associations between both BPD and severe ROP (grade  $\geq 3$ ) and higher risk of morbidity, including neurocognitive impairment in adult life (182, 183). Accordingly, preterm birth has become an important public health issue. Both suboptimal nutrition and poor growth during vulnerable periods of development have been associated with impaired neurodevelopmental outcomes in preterm infants (184-191). Poor postnatal growth caused by insufficient energy and macronutrient intakes has been associated with poor neurodevelopmental outcome (184).

The only RCT in which preterm infants received either unfortified DHM or premature formula without confounding by the mother's decision to breast feed was performed in the early 1980s (192). In a follow-up study of Singhal et al, blood pressure (193) and plasma lipoprotein profile (194) were measured at the age of 13-16 years, in 23 % of a cohort of 926 children who were born prematurely and had participated at birth in two parallel randomised trials in five neonatal units in the UK. Lower mean blood pressure and more favourable plasma lipid profile with a lower ratio of low-density to high-density lipoprotein cholesterol compared to those fed with premature formula, were seen in adolescents who had randomised to receive DHM during neonatal period, either as sole diet or a supplement to MOM. In contrast to the hypothesis that rapid early growth may influence later metabolic risk factors (195), Henriksen et al found no associations between rapid weight gain during the first year of life and metabolic markers, such as cholesterol, fatty acids, insulin growth factor 1, adiponectin, leptin and HbA1c (196).

## 2 OBJECTIVES

The overall objective of this thesis was to increase knowledge regarding extremely preterm infants' nutritional intakes over time and during the perioperative week surrounding surgical treatment for PDA. This thesis focuses on early and perioperative nutrition and investigate implemented changes and associations with growth, BPD and ROP as well as differences between NICUs regarding perioperative nutrition.

Specific objectives of the included studies were:

### Paper I

To describe interventions regarding nutritional intakes in Stockholm, Sweden between 2004 and 2011 and to investigate if energy and macronutrient intakes in and growth of extremely preterm infants have increased over time.

### Paper II

To investigate if higher energy- and protein intake the first week of life and the first month of life were associated with reduced initial weight loss and reduced risk of ROP and BPD in extremely preterm infants, respectively.

### Paper III

To describe perioperative nutrition, including energy-, macronutrient- and fluid intakes, during the week surrounding ductal ligation for PDA in extremely preterm infants born in Sweden between 2004 and 2007.

### Paper IV

To describe differences in perioperative nutrition between Swedish NICUs during the years 2004 to 2007.

## 3 METHODS

### 3.1 STUDY POPULATION AND DATA COLLECTION

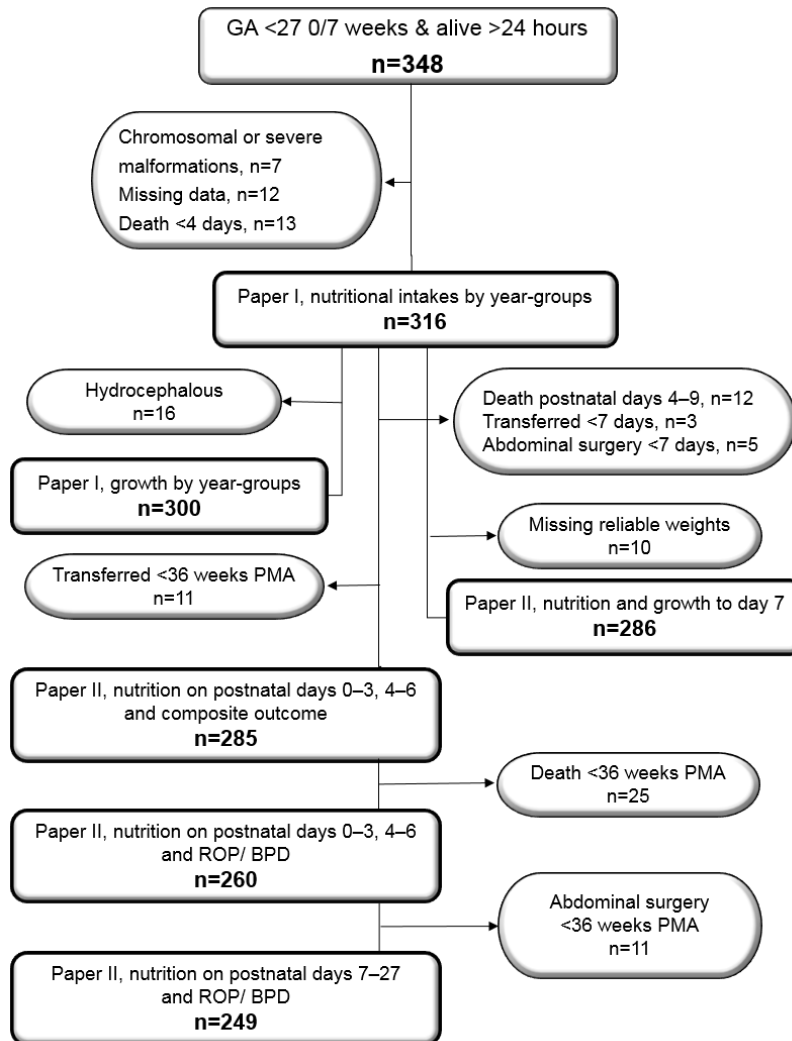
All four papers in this thesis are retrospective observational studies.

Overview of the thesis' four papers				
Paper	Research question	Exposure	Outcomes	Statistics
<b>I</b>	To investigate if nutritional interventions improved energy and macronutrient intakes as well as growth.	Nutritional interventions including e.g., guidelines update, aggressive nutrition, ward staff education, ready-to-use PN solutions.	Energy- and macronutrient intakes, growth.	Descriptive statistics, quantile regression with bootstrapped confidence intervals.
<b>II</b>	To investigate association of higher energy- and protein intake during the first week and month of life related to growth and morbidities.	Energy- and protein intake measured during the first week and month of life.	Growth as well as occurrence of composite (death or BPD) and ROP/BPD.	Multiple variable adjusted linear regression, Poisson regression.
<b>III</b>	To investigate nutritional intakes during the week surrounding day of PDA surgery.	Perioperative week starting 3 days prior and ending 3 days post-surgery	Energy-, macronutrient- and fluid intakes.	Descriptive statistics, one way ANOVA with Bonferroni.
<b>IV</b>	To investigate differences between NICUs regarding nutritional intakes during the week surrounding day of PDA surgery.	Five Swedish NICUs.	Energy-, macronutrient- and fluid intakes.	Descriptive statistics, one way ANOVA with Bonferroni.

### 3.2 STUDY POPULATION

In all four studies infants born in Sweden at less than 27 postnatal weeks between 2004 and 2011 and who survived >24 h, were included. In papers I and II, the study period comprised eight and four weeks, respectively. In paper II, infants were excluded if abdominal surgery or

transfer outside Stockholm occurred before the first outcome, e.g., before postnatal day seven, Figure 8. In papers III and IV, the study period comprised one week surrounding ductal ligation for PDA. In all papers, infants with major chromosomal or severe congenital anomalies were excluded as well as infants with incomplete nutritional data or missing data from the hospital records.



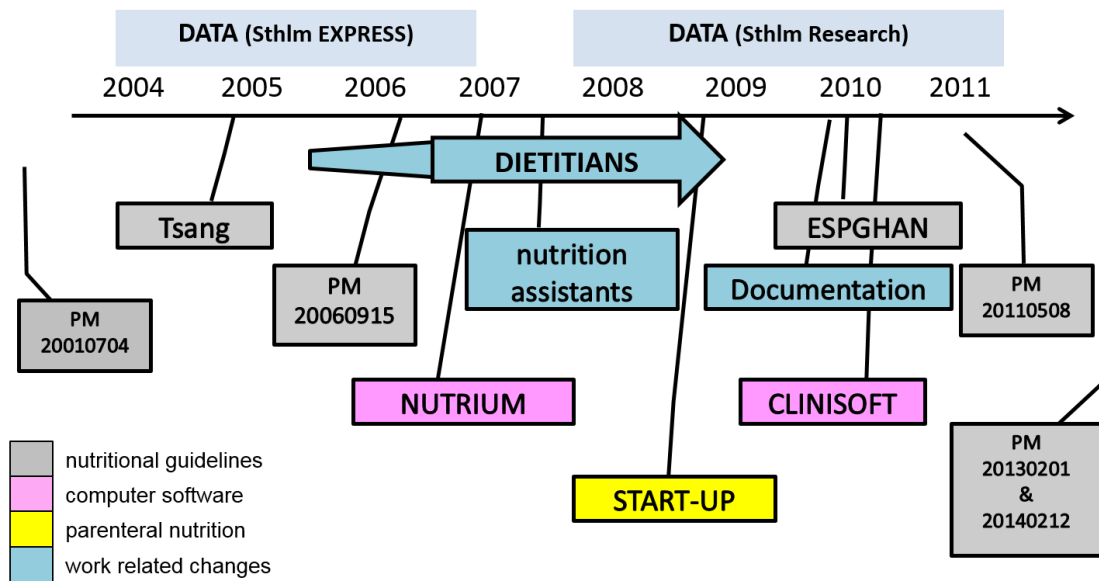
**Figure 8.** Flowchart of infants included in Paper I and II.

### 3.2.1 Papers I and II

#### *Paper I*

The Stockholm-study consists of the Stockholm section of The EXPRESS cohort (2004-2007) and Stockholm research study (2008-2011).





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**Figure 9.** Interventions affecting nutritional intakes between 2004 and 2011. PM (gray) denote local guidelines.

The interventions that occurred during the study period were: Update of international and local nutritional guidelines, introduction of computer software facilitating nutrition evaluation and prescription and introduction of ready-to-use parenteral solution simplifying early start of protein and carbohydrates, Figure 9. Work related interventions were: Increased employment of dietitians, upgraded status for the milk kitchen staff to nutrition assistants and implementation of methods for documentation, as well as changes in nutritional practice regarding detailed PN and EN, Table 2. Energy- and macronutrient intakes as well as growth were investigated over eight-year period stratified by four year-groups. Infants eligible for inclusion were 348 and infants included in the final nutrition and growth analyses were 316 and 300, respectively, Figure 8.

**Table 2.** Interventions in nutrition practices.

<b>Parenteral nutrition</b>	<b>Start postnatal day</b>	<b>Minimal and maximum macronutrient supply g/kg/d</b>	<b>Postnatal day for optimal macronutrient supply</b>	<b>Termination of parenteral fat infusion</b>
2004: International guidelines, 105-115 kcal/kg/d (197)	1	0.5 to 3.0-3.5 protein 0.5 to 2.5-3.0 fat	Minimal 6-7 days	60 to 75 % provided as enteral nutrition
2006: Local guidelines based on international guidelines, 105-115 kcal/kg/d (17)	0-1	2.0 to 4.0 protein 1.0 to 3.5 fat	2-3 days regarding protein 3-4 days regarding fat	60 to 75 % provided as enteral nutrition
2011: Local guidelines based on international guidelines, 105-115 kcal/kg/d (17)	0	2.0 to 4.0 protein 1.0 to 3.5 fat	2 days regarding protein 3 days regarding fat	75 % provided as enteral nutrition
<b>Enteral Nutrition</b>	<b>Start of breast milk fortification</b>	<b>Maximum energy- and macronutrient supply, g and kcal/kg/d</b>	<b>Postnatal day for optimal intakes</b>	<b>Comments</b>
2004: International guidelines (197)	100 % enteral nutrition	3.8 g protein for infants weighing < 1000g 6.6 g fat	4 to 14 days	Non aggressive feeding advancement strategy
2006: Local guidelines based on international guidelines (17)	100 % enteral nutrition	4.0 g protein 6.6 g fat 150 kcal	4 to 14 days	Idem Change of protein fortifier from whole cow milk to extensively hydrolysed
2010: International guidelines (18)	75 % enteral nutrition	4,5 g protein for infants weighing < 1000g 8.4 g fat 135 kcal	2 to 7 days	Aggressive feeding advancement strategy Adherence to the international guidelines occurred before publication

## *Paper II*

The Stockholm-study consists of the Stockholm section of The EXPRESS cohort (2004-2007) and Stockholm research study (2008-2011) with 348 eligible infants. Energy, protein (inclusive PE ratio) and fluid intakes as well intakes of transfusions were measured during early neonatal and late neonatal period. Outcome measurements were, growth as well as the occurrence of composite death or BPD and ROP/BPD. Analyses during the early neonatal period (0-6 days) regarding nutrition to growth, to composite death or BPD, and to ROP/BPD and during the late neonatal period (7-27 days) regarding nutrition to ROP/BPD were performed for 286, 285, 260 and 249 infants, respectively, Figure 8. Figure 10 a and b, show dietitian assistants in action in the ward kitchen.



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**Figure 10 a.** Dietitian assistants in the ward kitchen preparing individually fortified breast milk.



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**Figure 10 b.**

### 3.2.2 Papers III and IV

#### *Paper III*

The subset of The EXPRESS cohort (2004-2007) with 141 eligible infants. The perioperative days were measured during one week starting 3 days prior to PDA ligation and ending after 3 days post-surgery. Outcome measurements were, energy-, macronutrients- and fluid intakes per day/week. Analyses during perioperative week were stratified by gestational age and neonatal period in which surgical treatment was performed as well as compared to minimal estimated nutritional needs according Tsang et al (17) and Agostoni et al (18). Due to unobtainable nutritional data, one infant was excluded and finally 140 infants were included in the study.

#### *Paper IV*

The subset of The EXPRESS cohort with 141 eligible infants. The perioperative days were measured during one week starting 3 days prior to PDA ligation and ending after 3 days post-surgery. Outcome measurements were, energy-, macronutrients- and fluid intakes per day and week. Differences of nutritional intakes during the perioperative week were investigated between five Swedish NICUs. Data were stratified by neonatal period in which surgical treatment was performed, type of fluid (EN, PN) and compared to estimated nutritional requirements. Due to unobtainable nutritional data, one infant was excluded and finally 140 infants were included in the study.

### 3.3 DATA COLLECTION

For all studies, detailed nutritional data retrieved from hospital records and retrospectively registered in a nutritional software program ([www.nutrium.se](http://www.nutrium.se), Nutrium AB, Umeå, Sweden), included: All fluid intakes containing EN and PN, MOM, DHM, HMF, vitamin and mineral supplements, blood products, glucose infusions including electrolytes and anthropometrical data, Figure 11. The data-base comprised eight weeks of nutritional data regarding papers I and II, of which weeks one to four and five to eight were on daily (days 0-28) and weekly (day 35, 42, 49, 56) basis, respectively. Regarding papers III and IV, the data-base comprised one week's (7 days) nutritional data. In total, 12116 (papers I, II = 11136, papers III, IV = 980) postnatal days were registered in Nutrium. Data registration turned out to be very time consuming. It took up to four hours or more to register one infant in Nutrium.

Nutrient ▲		Intag (/kg/d)
Vätska		137 ml
Energi		78 kcal
Protein / a.a.		2.13 g
Kolhydrater		12.4 g
Glukos		8.2 mg/kg/min
Fett		2.32 g

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**Figure 11.** Calculation of energy (kcal/kg/d), macronutrients (g/kg/d) and fluid intake (ml/kg/d) in Nutrium, regarding one study infant. Red bars to the left of the middle line denote largely suboptimal intakes of energy and protein.

In papers I and II nutritional data of all postnatal days during the eight postnatal weeks were used in the analyses. Missing data are presented in flowchart, Figure 8. In paper II, protein intake was evaluated as PE ratio, calculated as the fraction of the protein intake (g/kg/d) per 100 kcal of energy intake (kcal/kg/d), if intake of energy was significantly associated with the outcomes. Nutritional intake of blood products was included in both papers II and III. Energy and protein estimated contents were 18 kcal/100 ml and 4.1 g/100 ml and 28 kcal/100 ml and 6.9 g/100 ml regarding erythrocytes and plasma or thrombocytes, respectively (198, 199). In papers III and IV data included seven days during perioperative week starting three days prior and ending three days post-surgery for PDA. Of 980 possible days twelve were missing because of death (n=1, 2 days), incomplete nutritional data (n=3, 9 days) and transfer to county hospital (n=1, 1 day). Relevant perinatal data were retrieved from Swedish neonatal quality (SNQ) register and hospital records. Questionnaires inquiring nutritional practice during perioperative week were sent to NICUs, included in paper IV.

Mid-infrared spectrophotometry was used to determine macronutrient content in HM (MilkoScan 4000, FOSS Hillerød, Denmark) at Eurofins Steins Laboratory AB, Jönköping, Sweden (48). MOM not analysed was equalled to the average content of the analysed MOM expressed from mothers of EPT infants before or after 28 postnatal days and used in nutritional calculations (48). DHM not analysed was equalled to the average content of analysed mature (> 28 days) HM samples.

### 3.4 STATISTICAL METHODS

Levene's test was used to investigate the distribution of continuous data. The hypothesis of normality was rejected when the p-value < 0.05. If the test of normality failed, it allowed you to state with 95 % confidence, that the data did not fit normal distribution. However, passing the normality test only said that no significant deviation of normality was found.

### **3.4.1 Paper I**

Data were analysed in periods of two years (year-groups). To best illustrate data, median, 10<sup>th</sup> to 90<sup>th</sup> percentiles were used. Differences in nutritional intakes were examined using quantile regression with bootstrapped confidence intervals (200). Mixed effects model including restricted cubic splines were used to examine differences in growth (201, 202).

### **3.4.2 Paper II**

Linear and Poisson regression with robust standard errors to study the outcome of interest was used (203). Associations with protein intake was evaluated as PE ratio (interaction term), if energy intake was significantly associated with outcome. If there was no measurement on postnatal day seven, that day's weight was calculated assuming an exponential growth pattern between measurements within two weeks, according to Patel et al (204).

Both risk for any ROP and severe ROP were examined when there was an association with risk of ROP. Differences in relative risk were calculated and expressed as risk ratios. To include predictors in the regression models, directed acyclic graphs were used as selection tool. Predictors as antenatal corticosteroids, GA in days, birth weight standard deviation score (BWSDS), sex, fluid intake, transfusions of erythrocytes and plasma, and days of MV were considered as potential confounders. Natural cubic splines were used to test linearity in the models, according Durrleman and Bassett (201). When the interaction term between MV and fluid intake was significant, potential interaction in the association between exposure and outcome was tested and supplemented with stratified analyses. Adjustments for period of birth's analyses were also reported. An interaction term between energy intake and time period was included to test differences in association between time periods as time period (2004-2007 and 2008-2011) was included as a factor variable.

### **3.4.3 Papers III and IV**

Descriptive statistics were presented in mean (SD), median, interquartile range (IQR) and numbers (%). One-way between-groups ANOVA with post-hoc tests was used to demonstrate differences regarding energy-, macronutrient and fluid intakes between perioperative days and NICUs (independent grouping variable with more than two levels). One-way ANOVA showed whether there were significant differences between the groups and post-hoc tests were used to identify the exact differences. Since intakes of EN and PN were not normally distributed, the non-parametric Kruskal-Wallis Test was used to show differences between NICUs regarding intakes of EN and PN.

## 4 RESULTS

This section comprises the summary of the results of the four studies included in this thesis with the Stockholm section of the EXPRESS cohort and the Stockholm research study (Papers I and II) as well as the sub-cohort of EXPRESS study (Papers III and IV)

### 4.1 INFANT CHARACTERISTICS

Weight at birth was measured in 456 infants (316 in paper I and II, 140 in paper III and IV). Of all 489 (n=348 + n=141) infants, 50 died before 36 postnatal weeks of which 13 infants died <4 postnatal days, 12 infants died between postnatal days 4 and 9 and one infant died on postnatal day 16 (on day + 1 after PDA surgery). Of 456 infants 29 were born small for gestation (15 in paper I and II and 14 in paper III and IV). In Table 3 baseline and background characteristics are presented.

**Table 3.** Infant characteristics at birth.

	<b>Paper I-II</b>	<b>Paper III-IV</b>
Time interval, postnatal days	28-56	7
Number of infants	316	140
Gestational age, weeks	25.3 $\pm$ 1.1	24.8 $\pm$ 1.0
Birth weight, g	784 $\pm$ 166	723 $\pm$ 156
Birth length, cm	32.7 $\pm$ 2.4 (n=288)	32.0 $\pm$ 2.5 (n=102)
Birth HC, cm	23.2 $\pm$ 1.5 (n=295)	22.8 $\pm$ 1.6 (n=108)
Sex, male, n (%)	169 (53)	83 (59)
Apgar score		
1 minute	4.9 $\pm$ 2.4	4.8 $\pm$ 2.6
5 minutes	7.0 $\pm$ 2.3	7.1 $\pm$ 2.0
10 minutes	8.4 $\pm$ 1.7	8.2 $\pm$ 1.5
CRIB score	8.9 $\pm$ 4.5 (n=198)	7.7 $\pm$ 3.5 (n=136)

Data are mean  $\pm$  SD values or numbers (%)

### 4.2 HIGHLIGHTS

#### Paper I

After exclusion of 32 infants due to chromosomal anomalies or severe malformations, missing data, death <day 4, 316 infants were included in this paper, Figure 8.

## Highlights

Energy and macronutrient intake improved over time.

The amount of enteral nutrition was highest during the two-year period of 2008-2009.

During the last two-year period (2010-2011), energy and protein intake approximately met minimal estimated needs, according to Swedish guidelines.

Growth during later years improved compared to the first two-year period (2004-2005).

## Paper II

After exclusion of 52 infants due to chromosomal anomalies or severe malformations, missing data, death <day 10, transferred <day 7 and abdominal surgery <day 7, 296 infants were included in this paper, Figure 8.

## Highlights

Increased energy and protein intake during the first week reduced initial growth restriction. Increased energy intake during the first month of life is associated with lower risk for BPD and ROP.

Increased energy and protein intakes during the first month of life are associated with lower risk for BPD for infants born during the years 2008-2011.

The effect of increased nutritional intake and risk for ROP is less when mechanical ventilation is needed.

## Paper III

After exclusion of one infant due to unobtainable data, 140 infants were included in this paper.

## Highlights

Perioperative nutrition in Sweden is suboptimal compared to minimal estimated nutritional needs.

Perioperative nutritional intakes of infants who received ductal ligation during early neonatal period are lower compared to those ligated during late neonatal and postneonatal periods.

## Paper IV

After exclusion of one infant due to unobtainable data, 140 infants were included in this paper.

## Highlights

Perioperative nutrition differs between Swedish NICUs.

Fluid intake and distribution between enteral and parenteral nutrition differ largely between NICUs.

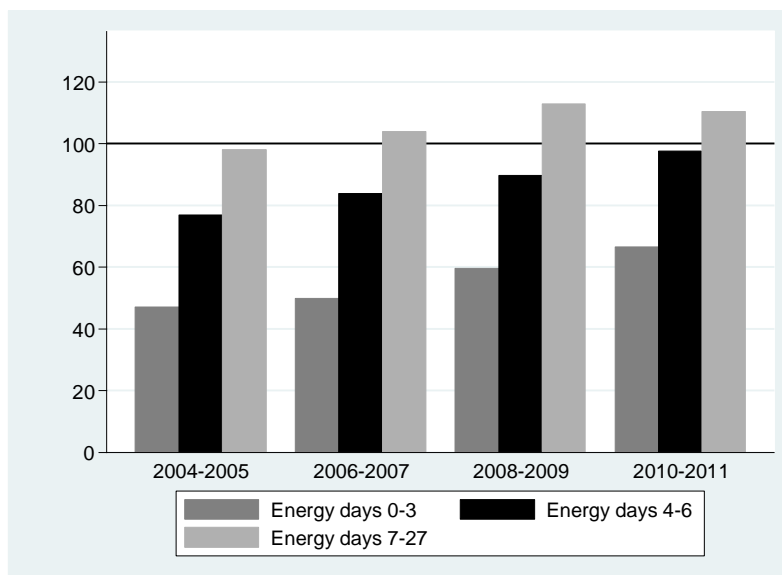


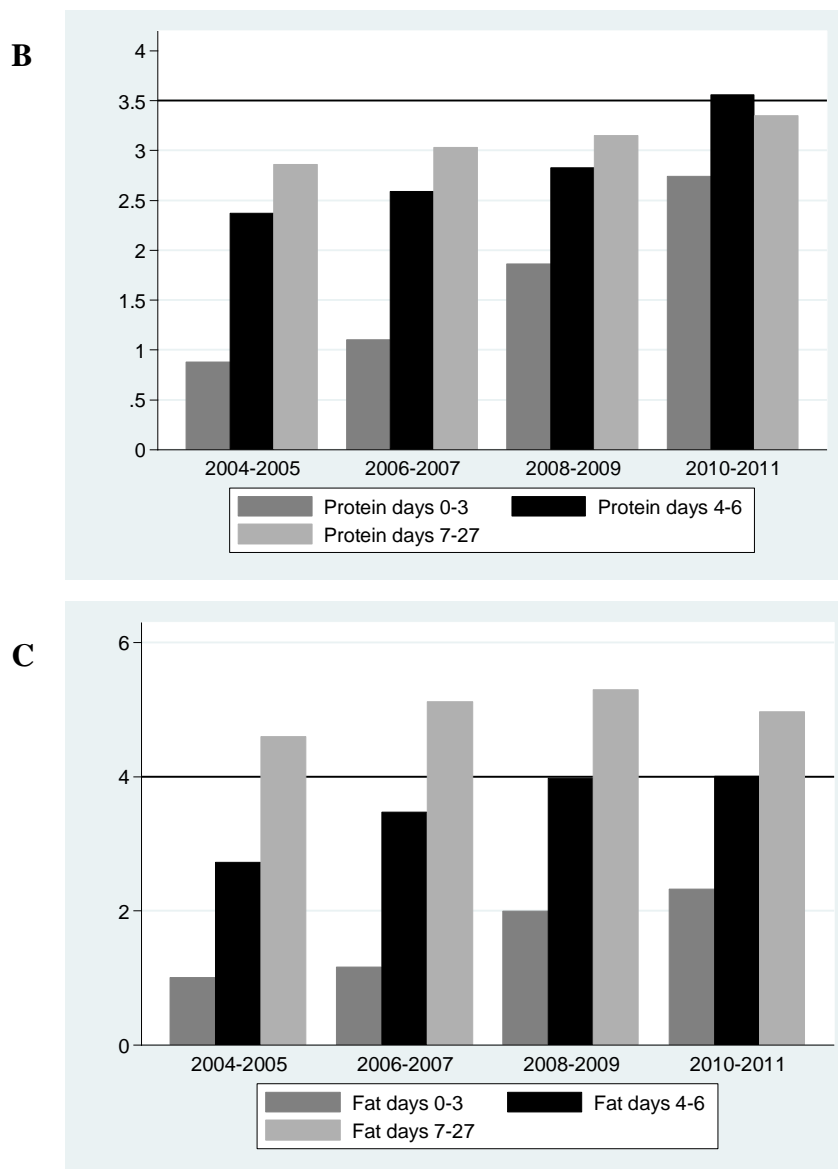
#### 4.2.1 Papers I and II, improved nutrition and growth

Energy, protein and fat intakes during the first postnatal week (days 0-6) improved over time, while differences regarding intakes between year-groups during days 7-27 were smaller, Figure 12 A, B, C. Protein intakes during the first postnatal week (days 0-6) and subsequent weeks (days 7-27) improved during the years but in year-group 2010-2011, only the protein intake during later part of the first postnatal week (days 4-6) met the minimal estimated needs for postnatal day four, according to the Swedish guidelines, Figure 12 B (20).

Difference in median energy-, protein- and fat intake during days 0-3 between year-groups 2004-2005 and 2010-2011 was 19 kcal/kg/d (95 % CI:16-22,  $p < 0.001$ ), 1.9 g/kg/d (95 % CI:1.6-2.1,  $p < 0.001$ ), 1.3 g/kg/d (95 % CI:1.2-1.5,  $p < 0.001$ ), respectively, Figure 11 A, B, C. Difference in median energy-, protein- and fat intake during days 4-6 between year-groups 2004-2005 and 2010-2011 was 21 kcal/kg/d (95 % CI:16-27,  $p < 0.001$ ), 1.2 g/kg/d (95 % CI:1.0-1.4,  $p < 0.001$ ), 1.3 g/kg/d (95 % CI:1.0-1.6,  $p < 0.001$ ), respectively. Difference in median energy-, protein- and fat intake during days 7-27 between year-groups 2004-2005 and 2010-2011 was 12 kcal/kg/d (95 % CI:6-18,  $p < 0.001$ ), 0.5 g/kg/d (95 % CI:0.4-0.6,  $p < 0.001$ ), 0.2 g/kg/d (95 % CI:-0.6-0.9,  $p = 0.61$ ), respectively.

A





**Figure 12.** Median intakes of energy (A), protein (B) and fat (C) during postnatal days 0-3, 4-6 and 7-27, stratified by year-groups. The black lines denote minimal estimated needs for postnatal day four, according to Swedish guidelines (20).

The proportion of enteral feedings during days 0-6 was greater in year-group 2008-2009 compared to year-group 2010-2011 and difference was 6.7 % (95 % CI: 2.2-11.2,  $p < 0.004$ ). The feeding advancement in year-group 2008-2009 was faster compared to all other years and median time to full feeds was 15 (8-34) days. In contrast, median time to full feeds was 23 (10-41) days in year-group 2010-2011. Nevertheless, higher total energy intake during days 7-27 compared to all other years was demonstrated in year-group 2010-2011 in infants receiving more than 50 % enteral feedings, but not full enteral feedings. This significant difference was due to a higher energy intake of PN and the difference lost the significance when more than 75 % of the total fluids were provided as enteral feedings.

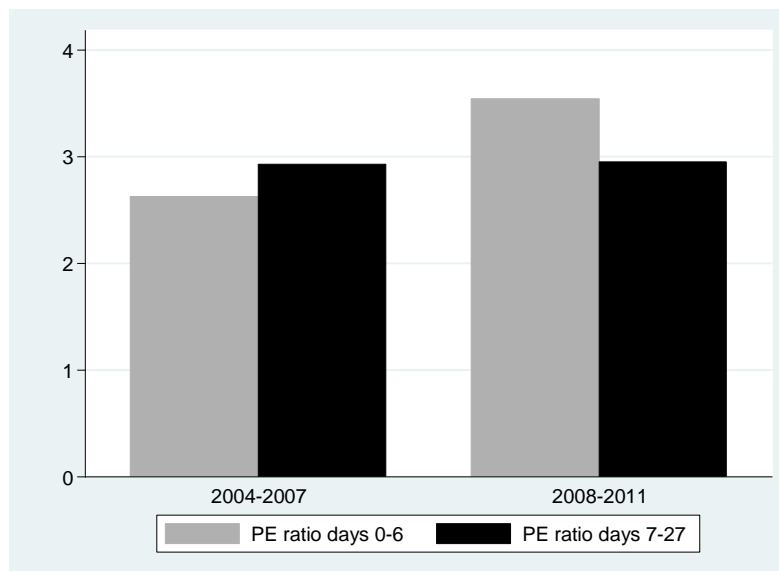
Growth improved significantly during later years compared to year-group 2004-2005. At postnatal days 7 and 56, the weight growth difference ( $\Delta$ WSDS) was 0.3 SDS (95 % CI: 0.1-0.5,  $p<0.001$ ) and 0.4 SDS (95 % CI: 0.2-0.7,  $p<0.001$ ), respectively.

In paper II, higher energy- and protein intake during the first week of life reduced initial growth restriction. Every additional energy intake of 10 kcal/kg/d during days 4-6 was associated with 0.08 higher weight SDS (WSDS) on day 7 ( $p<0.001$ ), adjusted for GA, BWSDS, transfusions and days of MV. A higher PE ratio on days 4-6 was also significantly associated with initial weight development. Even though higher intakes of energy and protein on days 0-3 also were positively associated with  $\Delta$ WSDS to day 7.

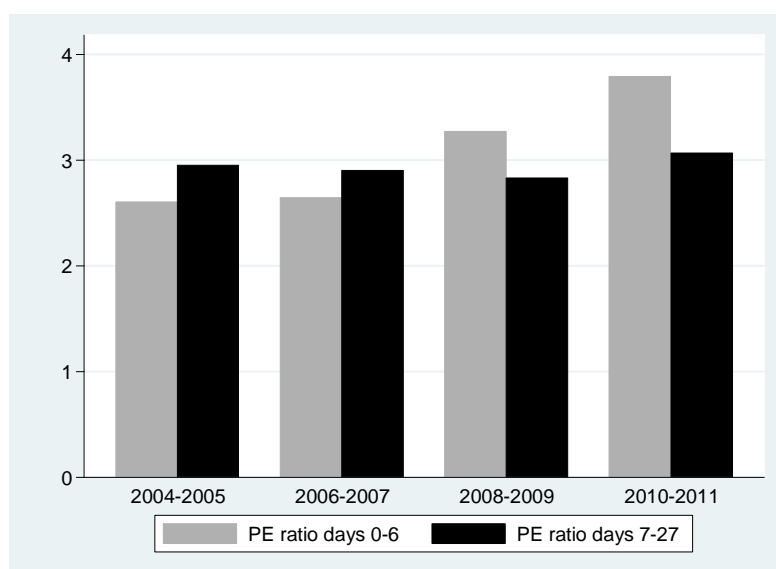
The PE ratio increased over time during the early neonatal period (days 0-6) from 2.6 g protein/100 kcal in the Stockholm section of The EXPRESS cohort (2004-2007) to 3.5 g protein/100 kcal in the Stockholm research study (2008-2011) ( $p<0.001$ ), Figure 13 A. During the late neonatal period (days 7-27) the difference in PE ratios between the two cohorts was 0.1 g protein/100 kcal.

The PE ratio during days 0-6 increased over time from 2.6 g protein/100 kcal in year-groups 2004-2005 and 2006-2007 to 3.3 g protein/100 kcal in year-group 2008-2009 and to 3.8 g protein/100 kcal in year-group 2010-2011 ( $p<0.001$ ), Figure 13 B. The PE ratio during days 7-27 decreased from 2.9 g protein/100 kcal in year-groups 2004-2005 and 2006-2007 to 2.8 g/100 kcal in year-group 2008-2009. In year-group 2010-2011, the PE ratio increased with 0.3 g protein/100 kcal compared to year-group 2008-2009 ( $p=0.001$ ), Figure 13 B.

A



B



**Figure 13.** PE ratios (g protein/100 kcal) with the exclusion of blood products during early (0-6 days) and late neonatal (7-27 days) period, stratified by cohorts (A) and by year-groups (B).

#### 4.2.2 Paper II, nutrition and morbidity

Mean protein intake excluding the blood products for the whole cohort was 1.8 g/kg/d on days 0-3, 2.9 g/kg/d on days 4-6 and 3.1 g/kg/d on days 7-27. With the inclusion of the blood products, protein intake increased by 1.1 g/kg/d, 0.5 g/kg/d and 0.3 g/kg/d, respectively for days 0-3, 4-6 and 7-27, respectively, compared to protein intake without blood products. When blood products were included, PE ratios were 4.6 protein g/100 kcal, 3.8 g protein/100 kcal and 3.1 g protein/100 kcal, respectively for days 0-3, 4-6 and days 7-27.

Association between 10 kcal/kg/d higher energy intake on postnatal days 7-27 and lower risk of BPD (9 %), adjusted for GA, BWSDS, antenatal corticosteroids, transfusions and mechanical ventilation (MV) was significant ( $p=0.029$ ). In infants with  $\leq 10$  days of MV, higher energy intake on days 7-27 was associated with reduced risk of BPD ( $p=0.011$ ), in analyses adjusted for GA, BWSDS, antenatal corticosteroids, transfusions and MV. The association between energy intake and BPD was not significant in infants with  $>10$  days of MV. Although higher intakes of both energy and protein on postnatal days 7-27 in infants with  $>10$  days on MV, reduced the risk of BPD. Increased PE ratio at a mean energy intake of 100 kcal/kg/d on days 7-27 was not associated with a significant reduction of BPD risk. However, every 0.5 g/kg/d increase in protein intake when energy intake equals 120 kcal/kg/d reduced the risk of BPD by 25 % ( $p=0.034$ ).

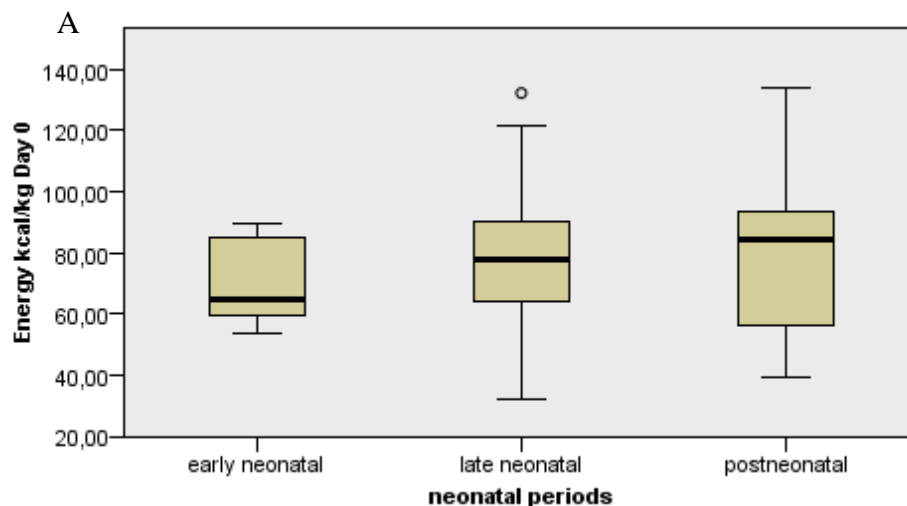
Association between 10 kcal/kg/d higher energy intake on postnatal days 7-27 and lower risk of ROP of any stage (6 %), adjusted for GA, BWSDS, transfusions and MV was significant ( $p=0.005$ ). No significant association was demonstrated between 10 kcal/kg/d increase on postnatal days 7-27 and lower risk of severe ROP. In infants with  $\leq 10$  days of MV, higher

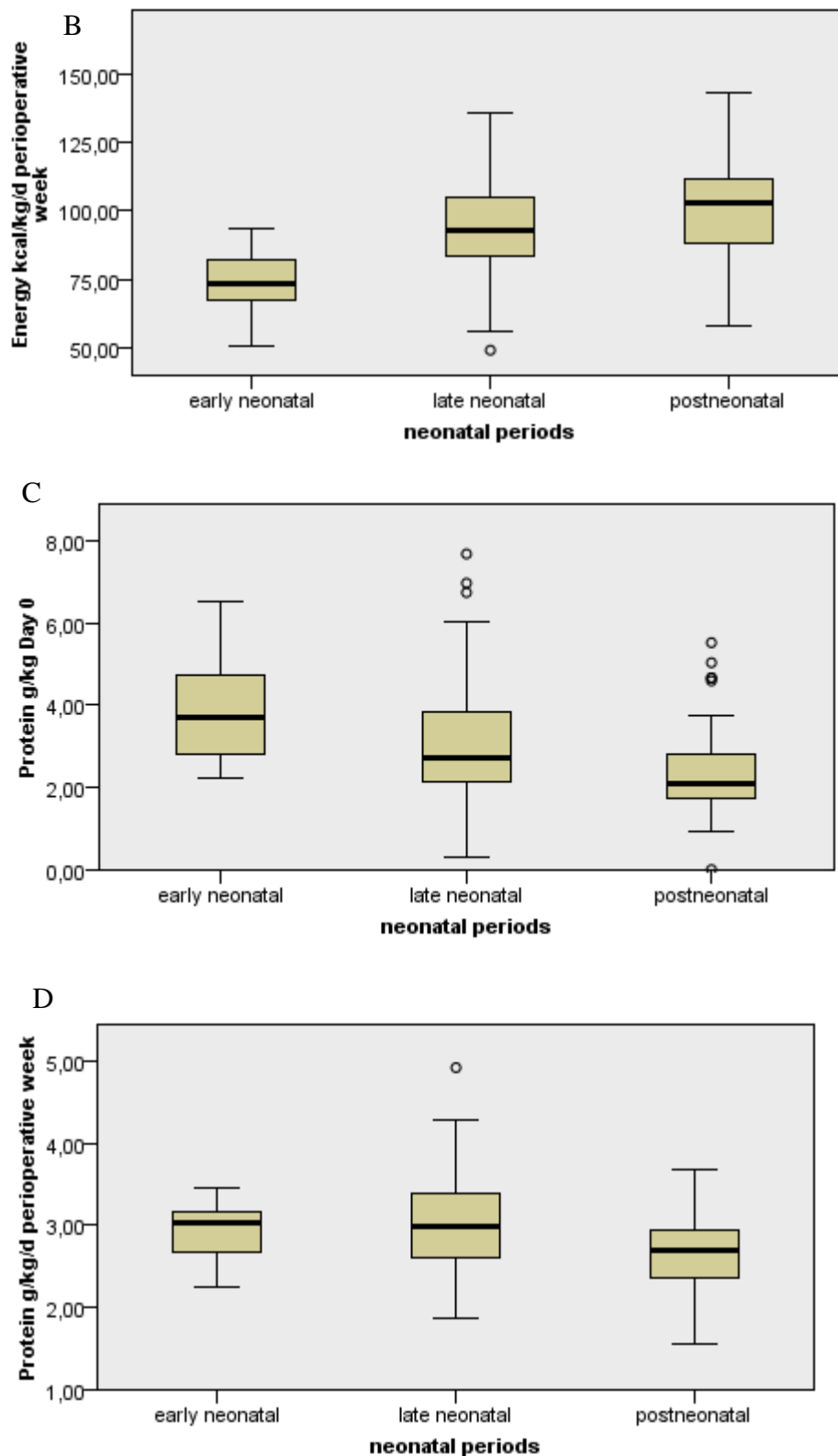
energy intake on days 7-27 was associated with reduced risk of ROP ( $p=0.003$ ) in adjusted analyses. When energy intake increased from 110 to 120 and 120 to 130 kcal/kg/d, the estimated absolute risk of ROP decreased with 10 % and 8 %, respectively. In addition, when the analyses were adjusted for year-groups, associations in infants with <10 days MV were significant (RR 0.89, 95 % CI 0.81-0.98;  $p=0.024$ ), while this was not the case in infants with >10 days MV (RR 1.01, 95 % CI 0.97-1.04;  $p=0.768$ ). The PE ratio on days 7-27 was not associated with risk of ROP in the adjusted analyses.

#### 4.2.3 Paper III, perioperative nutrition

Perioperative nutrition was suboptimal according to minimal estimated nutritional needs (110 kcal/kg/d, 4 g protein/kg/d, 4.8 g fat/kg/d and 12 g carbohydrate/100 kcal) and energy, protein, fat and fluid intakes on the third day post-surgery for PDA were still not improved compared to intakes three days prior ductal ligation.

Energy intake on the day of surgery (day 0) did not differ significantly between infants who underwent ductal ligation for PDA during the first 6 postnatal days (early neonatal period), postnatal days 7-27 (late neonatal period) or  $\geq 28$  postnatal days (postneonatal period), Figure 14 A. In contrast, energy intake during perioperative week improved for every following neonatal period (74 kcal/kg/d, 93 kcal/kg/d, 103 kcal/kg/d,  $p=0.001$ ), Figure 14 B. Protein intake during day 0 was highest in infants who underwent ductal ligation during the first 6 postnatal days (early neonatal period) and thereafter decreasing for every following neonatal period (3.7 g protein/kg/d, 2.7 g protein/kg/d, 2.1 g protein/kg/d,  $p=0.003$ ), Figure 14 C. Protein intake during perioperative week was equal in infants who underwent ductal ligation during the early and late neonatal periods (3 g/kg/d) and lower compared to those who underwent ductal ligation during the postneonatal period. The difference in protein intake between infants who underwent ductal ligation during the early/late neonatal periods and the postneonatal period was 0.3 g/kg/d ( $p=0.02$ ), Figure 14 D.





**Figure 14.** Data are median energy (kcal/kg/d) intakes during day of surgery = day 0 (A) and perioperative week starting 3 days prior to PDA ligation and ending after 3 days post-surgery (B), as well as protein (g/kg/d) intakes during day of ductal ligation = day 0 (C) and perioperative week starting 3 days prior ductal ligation for PDA and ending 3 days post ductal ligation (D), stratified by neonatal period. Early (days 0-6) and late (days 7-27) neonatal periods as well as postneonatal ( $\geq 28$  days) period denote the postnatal age in which ductal ligation took place. Energy- and protein intakes include nutritional content of blood products.

#### 4.2.4 Paper IV, perioperative nutrition, stratified by NICU

Differences between NICUs regarding nutritional intakes were observed and regarding total- and enteral fluid intakes as well as concentration of parenteral nutrition, differences were large. Furthermore, discrepancy between recommended concentration of ready-to-use solutions of parenteral nutrition (205) and the NICU supplying PN with the highest concentration, compared to other NICUs, was 42 % and 39 % regarding parenteral energy and protein concentration, respectively. Figure 15 shows an EPT infant who needs professional care.



© NICU Karolinska University Hospital

**Figure 15.** Extremely preterm infant in an incubator.

## **5 DISCUSSION**

### **5.1 GENERAL DISCUSSION**

The overall objective of this thesis was to study energy, macronutrient and fluid intakes of EPT infants treated in NICUs in Sweden during an 8-year period from 2004 to 2011. The general research questions were: i) to describe nutritional intakes over time in Stockholm and the impact on growth and morbidities such as BPD and ROP (paper I and II), and ii) to describe perioperative nutrition in EPT infants who underwent surgical treatment of PDA and investigate differences between Swedish NICUs regarding nutrition during the week surrounding PDA surgery.

The main findings of this thesis demonstrate suboptimal energy and macronutrient intakes in EPT infants in Sweden during the study period from 2004 to 2007. However, energy and macronutrient intakes increased in Stockholm over the period from 2004 to 2011, mean protein intake during postnatal days 7 to 27 did not meet minimal estimated nutritional needs at full enteral nutrition according to Swedish guidelines (20). Higher energy intake on postnatal days 7 to 27 was associated with a lower risk of BPD and ROP and an association was seen between higher protein intake and reduced risk of BPD when sufficient energy was provided. The findings also showed that energy intake in combination with protein intake had a positive impact on weight development during the first postnatal week.

Suboptimal perioperative supply of energy and macronutrients was demonstrated in EPT infants undergoing ductal ligation for PDA in Sweden, particularly on the day of surgery. Moreover, problem with suboptimal perioperative nutrition did not improve with GA and largest deficits were faced by infants that underwent ductal ligation during early neonatal period (days 0-6). Nutritional intakes differed between Swedish NICUs but particularly regarding fluid intake, the difference between NICUs was large. Distribution between enteral- and parenteral fluid intakes differed also highly between NICUs as well as parenteral energy- and protein concentrations.

### **5.2 ENERGY AND MACRONUTRIENT INTAKE**

During the first postnatal week, energy and protein intake improved over the 8-year period (206) but did not meet the minimal estimated nutritional needs for day 4, according to Swedish guidelines (20). In contrast, fat intake met these minimal needs already during the years 2008-2009, coinciding with higher enteral intakes. Figure 16 shows detailed information regarding an individualised PN composition.



Tillverkat den:	2018-09-13	Dygnsvolym (mL):	108.18
Användes före:	2018-09-20	Summa i förpackningen (mL):	208.18
Admin dag:	2018-09-13	Ord. av Dr.:	Bohlin
Order No:	154755		
Karolinska apoteket, Solna		Komposition:	
Water for Injection (SE)	88.49 ml	Vaminolac (SE)	79.7 ml
Glucose 50% (SE)	23.71 ml	NatriumKlorid 4 (SE)	1.22 ml
KaliumKloride 2 (SE)	2.22 ml	Glycophos (SE)	2.88 ml
Peditrace (SE)	1.04 ml	Heparin (SE)	0.83 ml
Calcium gluconate 10%	7.73 ml	Magnesium Labesfal	0.58 ml
Doserings per kilo kroppsvikt:			
Natrium	7.0 mM/Kg	Kalcium	1.2 mM/Kg
Kalium	3.0 mM/Kg	Magnesium	0.3 mM/Kg
Fosfat	2.1 mM/Kg	Klorid	6.4 mM/Kg
Osmolarity: 857.12 mOsm/L			
Påbörjad: .....			
Förvaras i kylskåp			

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**Figure 16.** Protein, carbohydrates and micronutrient content in individual composition of parenteral nutrition.

Protein accretion is occurring when energy supply is at least 120 kcal/kg/d (79, 80), however, the ideal PE ratio is not known (65). In our study, paper II, PE ratios (including blood products) of 4.6 protein g/100 kcal and 3.8 g protein/100 kcal on days 0 to 3 and 4 to 6, respectively (207), were higher when compared to different PE ratios suggested in recent literature ranging from 3.1 to 4.1 g protein per 100 kcal (18, 81). PE ratio on days 4 to 6 was significantly associated with initial weight development and there was an association between higher energy and protein intake and reduced risk for BPD, particularly during the later period (2008-2011) when higher energy and protein intakes were supplied (207).

Inappropriate energy and protein intakes reduce growth, however, higher energy intake may enhance the rate of increase in skinfold thickness, if protein intake is appropriate (208). The PE ratio in the diet impacts the ratio of lean body mass to fat mass in the gained weight. Fairly et al showed that higher PE ratio (3.2 g/100 kcal versus 2.6 g/100 kcal) did not influence the proportion of fat to lean tissue in gained weight (209). In a small RCT study, growth and body composition of VLBW infants were investigated by comparing different PE ratios (210). Infants in the two experimental groups received high energy and high protein diets with different PE ratios: 150 kcal/kg/d with 4.2 g protein/kg/d, corresponding to PE ratio of 2.8 g/100 kcal and 150 kcal/kg/d with 4.7 g protein/kg/d, corresponding to PE ratio of 3.1 g/100 kcal. Energy and protein intake corresponding to PE ratio of 2.8 g protein/100 kcal was sufficient to increase weight growth and accretion of lean body mass.

Longer duration of MV during the later time period (2008-2011) was occurring among a higher proportion of infants developing BPD. Regarding higher energy intake on postnatal days 4 to 6 and BPD or ROP, there was no statistical significant association, although our results were towards an association between higher energy intake on postnatal days 4 to 6 and lower risk of severe ROP. It is possible to make comparisons between different stages of ROP since the diagnosis is based on visualized differences in retinal vascularisation. The

diagnosis of BPD might not be a valid predictor of later pulmonary problems because of its less distinct character. Severe ROP, NEC, and severe intraventricular haemorrhage (although not BPD) were associated with postnatal growth restriction according to Griffin et al (211).

Perioperative nutrition is suboptimal and infants that underwent ductal ligation during early neonatal (0-6 days) period faced the lowest nutritional intakes (212). Noteworthy is that protein intake during day of ductal ligation and perioperative week was highest in infants ligated during early neonatal period and lowest in those ligated during post neonatal ( $\geq 28$  days) period, respectively. Accordingly, PE ratio including blood products decreased from 3.9 g protein/100 kcal to 2.7 g protein/100 kcal. Recent studies demonstrated associations between PDA surgery, white matter injury and brain damage, however, data on nutritional intakes were not reported (213, 214). Since significance of undernourishment for outcome in EPT infants undergoing ductal ligation is still unknown, further investigation is required. Moreover, optimal postnatal age for surgical ligation of PDA is still not known (152, 153) and now there is a trend towards mainly pharmacological treatment of PDA.

Perioperative nutrition differed largely between NICUs in Sweden, paper IV. NICU A had highest energy and fat intake as well as the highest proportion of enteral feeds, compared to all other NICUs. However, NICU A's energy intake did not meet minimal estimated nutritional needs of full EN, according Swedish guidelines (20).

### **5.3 FLUID INTAKE**

The proposed cause of a physiological initial weight loss after birth is reduction of extracellular fluid. A reduction of 0.8 SDS in weight to postnatal day 7 followed by growth parallel to the Fenton growth chart in healthy preterm infants was suggested by Rochow et al (112). During the first postnatal week, intake of energy and protein rather than fluid intake was important for weight development, paper II (207). This result shows that attentive nutritional care has the potential to reduce initial growth restriction.

A Cochrane review based on five randomised clinical trials assessed that a careful restriction of fluid intake, not allowing for dehydration could be expected to decrease the risks of BPD, PDA, NEC, intracranial haemorrhage and death (156). In this review, fluid intakes during early (0-6 days) and late (7-27 days) neonatal period, between 60 to 80 ml/kg/d and 122 to 150 ml/kg/d, respectively, were considered as restricted, while fluid intakes during early and late neonatal period, between 140 to 150 ml/kg/d and 169 to 200 ml/kg/d, respectively, were considered as liberal. In our Stockholm studies (206, 207), fluid intake during early neonatal period could neither be accounted as restricted nor as liberal. In contrast, in the national studies, fluid intakes in three of five NICUs could be considered as liberal ranging from 123 ml/kg/d to 132 ml/kg/d. During late neonatal period, fluid intakes during 2-year period of 2004-2005 could be considered as restricted (147 ml/kg/d), while fluid intakes during all other years neither were restricted nor liberal. In contrast, our national studies showed liberal

fluid intake in EPT infants who underwent ductal ligation during early neonatal period (212). Large difference was demonstrated between NICUs regarding fluid intakes and during late neonatal period, fluid intakes ranged from restricted at NICU B (123 ml/kg/d) to liberal at NICU E (187 ml/kg/d), paper IV.

Suboptimal nutrient concentration in PN requires increased fluid amounts to meet estimated nutritional needs. During the years 2004 and 2007 parenteral energy concentration ranged from less than 40 kcal/100 ml to approximately 60 kcal/100 ml, while parenteral protein concentration ranged from less than 1 g protein/100 ml to approximately 2 g protein/100 ml, paper IV. In 2009, a standardised, extemporaneously prepared parenteral nutrition “Start-up” was introduced to facilitate the early start of protein supply on nights, weekends, or holidays when individually prescribed parenteral nutrition could not be prepared (206). Concentration of “Start-up” is 2.7 g protein/100 ml and only 50 kcal/100 ml, since fat is not included in this parenteral solution. Aforementioned PN concentrations did not meet the recommended content in modern all-in-one-bags, containing 91 kcal/100 ml and 3.1 g protein/100 ml (205). The use of highly concentrated PN makes it easier to meet estimated nutritional needs without exceeding fluid prescriptions, despite the need of extra fluid supply because of medical infusions.

## **5.4 ACTION BUNDLES OF NUTRITIONAL CHANGES**

However, changes in nutritional management during the 8-year study period certainly occurred at all Swedish NICUs, they were studied in detail at NICUs in Stockholm, paper I (206). These changes regarded e.g., implementation of guidelines and update of local guidelines, introduction and implementation of computer software, introduction of new nutritional products and nutritional education of the ward staff.

### **5.4.1 Guidelines**

International guidelines regarding PN and EN (17-19, 197) were available during the 8-year study period (2004-2011) and in Stockholm local PN and EN guidelines were based on these guidelines. Adherence to the international guidelines could occur before publication and update of local guidelines, which e.g., was the case in 2010 when aggressive feeding advancement strategy including earlier introduction and faster advancement of HMF was implemented, paper I (206). Clinical practice varied between Swedish NICUs, which already was noted during data acquisition regarding Nutrium-EXPRESS study (99, 215) and later on demonstrated in Paper IV.

Most guidelines are for healthy, growing preterm infants weighing >1000 grams as they are based on older studies performed of infants with birth weights between 1500 and 2000 grams (216, 217). However, in 2014, The National Board of Health and Welfare published

Swedish national guidelines (20), based on previous international guidelines but also on results of recent studies performed on EPT infants (99, 212). To meet the recommendation of postnatal day four including 50 % EN of the total fluid intake, according to Swedish guidelines constitutes a challenge for the ward staff. Differences between recent nutritional guidelines (20, 64, 65) and those from the last decades (17-19, 184, 194) are small, regarding e.g., energy- and macronutrient intakes, however, Tsang et al (17) recommended enteral energy intake of 150 kcal/kg/d with chronic lung diseases considering higher energy expenses. Recent parenteral nutritional guidelines regarding critically ill children at the paediatric intensive care unit recommend considering withholding macronutrients in PN for one week, while supplying micronutrients (65).

Studies have demonstrated improved nutritional intake and better postnatal growth in preterm infants, when enhanced nutrition protocols were implemented (218, 219). Despite achieving energy and protein intakes close to the target levels according guidelines, Saenz de Pipaon et al demonstrated difficulties in meeting intrauterine growth rates (121). Improved growth over subsequent years compared to the period, between 2004 and 2005 was demonstrated in our study, paper I (206), but infants still did not reach the intrauterine growth curve.

#### **5.4.2 Lack of guidelines**

Even though progress has been made during the last decades regarding neonatal nutritional needs, suboptimal nutrition is frequently occurring at NICUs, despite the knowledge that undernourishment contributes to mortality, morbidity and poorer developmental outcome (186, 220).

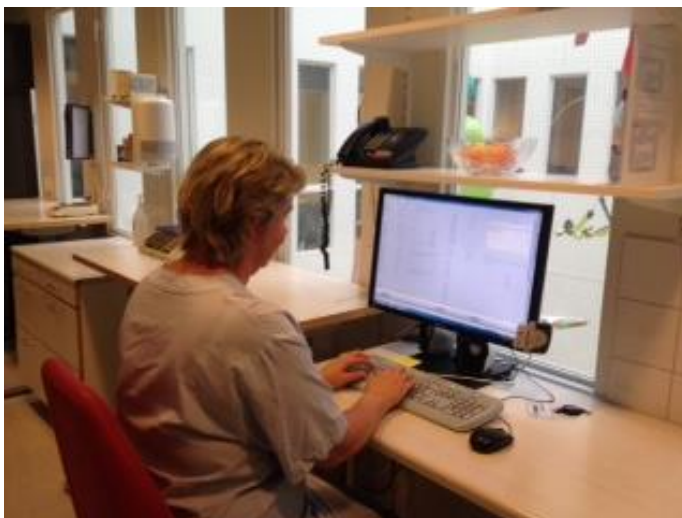
To our knowledge, nutritional guidelines for specific neonatal morbidities or treatments such as e.g., ductal ligation are lacking in literature. Suboptimal perioperative nutrition did not improve with GA, which could indicate that the underlying causes may reflect practices or guidelines, and not constraints related to GA, paper III (212). Enteral intolerance or limited vascular access in the most immature infants are some of the constraints related to GA. Moreover, optimal perioperative feeding strategies regarding term born infants with congenital heart disease are unknown, which may cause large variations in perioperative feeding practise between different hospitals (221).

Lack of guidelines regarding feeding intolerance and the ward staff's anxiety over infants developing NEC may lead to suboptimal nutritional intakes. Consensus regarding feeding intolerance parameters such as gastric residuals of prior feeding, bile or blood stained residuals, emesis, abdominal distention/tenderness and change in stool pattern/consistency has to be reached to prevent unnecessary withholding of feeds affecting nutritional intakes. In study context, reliable results are largely depending of careful defined criteria for feeding intolerance and its adherent application (170). At the NICU, optimal infrastructure and

designated ward staff are warrant for improved adherence to nutritional guidelines (220). Furthermore, achieving adequate nutritional intake constitutes a challenge during the transition phase from parenteral to enteral nutrition. Important reasons for difficulties in reaching target intakes could be, as before mentioned, lack of consensus on indications for withholding feeds and timing of the introduction of HMF. Faster advancement of daily increments ( $>24$  ml/kg/d) together with use of HMBFs might prevent the energy slope during the transition phase from PN to EN (169).

#### 5.4.3 Changes in clinical practice

Facilitating software, enhanced focus and education of personnel during the 8-year period had a combined impact leading to increased nutritional intake in EPT infants at the Stockholm NICUs. Although the tradition of performing nutritional calculations on an individual basis according to analysed HM was present before in Stockholm, the entry of Nutrium facilitated nutritional processes as well as engagement and education of the ward staff in nutritional issues. Wackernagel et al demonstrated the effect of computer software to facilitate prescriptions and documentation of nutritional intake in preterm infants (222). A supplemental computerised ICU system (Centricity Critical Care Clinisoft, GE Healthcare), registering all PN and medications as well as EN including fortification of MOM and DHM, facilitated the supervision of the nutritional process at NICUs in Stockholm (206). Documentation in the ICU system on a daily basis of fortified MOM and or DHM/premature formula prepared in the ward kitchen was performed by nutrition assistants, Figure 17.



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**Figure 17.** Dietitian assistant in the ward kitchen documenting individually fortified breast milk in the computerised ICU system.

In a recent study, Rochow et al observed that growth and nutrition comprised a greater part in the daily medical discussion after the introduction of an electronic pre-structured prescription ordering system giving day-by-day instructions for nutrition on an individual basis (108).

## 6 CONCLUSIONS

- Updated guidelines and their adherent application together with introduction and implementation of computer software, introduction of new nutritional products, intensification of nutritional education of ward staff contributed to improved nutritional intakes for EPT infants cared for in Stockholm.
- Energy and protein intakes during the first four postnatal weeks increased in EPT infants in Stockholm during the 8-year period.
- Improved energy and protein intakes during the first postnatal week were associated with reduced initial growth restriction.
- Improved nutritional intakes were associated with reduced occurrence of BPD and ROP.
- Perioperative nutrition in EPT infants undergoing ductal ligation was suboptimal compared to estimated nutritional requirements in Sweden.
- Total fluid, parenteral and enteral intakes differed largely between Swedish NICUs as well as the concentration in PN.
- The overall results from this thesis conclude that there are associations between higher nutritional intakes and increased growth and decreased risk for morbidity. Furthermore, nutritional intakes during perioperative week do not meet estimated nutritional requirements and differ between NICUs. There is room for improvement regarding nutritional intakes for EPT infants in general and during the week surrounding ductal ligation in particular.

## 7 FUTURE PERSPECTIVES

- Despite our knowledge, we still do not know the optimal preconditions regarding nutrition and growth nor what optimal nutrition and growth should look like and more research is needed in these vulnerable infants.
- Closer collaboration between dietitians, nutrition assistants, nurses, neonatologists, speech therapists and when surgical treatments are involved, anaesthesiologists and paediatric surgeons is necessary to increase awareness of the risk of undernutrition in EPT infants and further improve nutrition in the ward.
- Improvements of heat treatment and optimisation of HM fortification, regarding milk processing in HMB, should be focus of research. Furthermore, the potential clinical benefits of processed and fortified DHM need to be investigated.
- Increased resources are required to meet the follow-up needs of the vulnerable infant and their families, since many of infants born at the lower limit of viability are affected of undernutrition and neurodevelopmental disability.
- The target of the research should be that more EPT infants face a healthy future. Figure 18 shows healthy twins on return visit at the NICU.



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**Figure 18.** Twins born in gestational week 25 and three days. On the photograph, they are five months corrected age.



## 8 SVENSK SAMMANFATTNING

Normalt varar en graviditet ungefär 40 veckor. I Nordeuropa är antalet barn som föds prematurt, d v s före 37 graviditetsveckor, lägre än i andra regioner eller länder. Prematur födsel kan indelas i tre kategorier: Måttligt prematur födsel (32 till <37 graviditetsveckor), mycket prematur födsel (28 till <32 graviditetsveckor) och extremt prematur födsel (<28 graviditetsveckor). Av cirka 100 000 barn som föds varje år i Sverige är ungefär 1 % födda före graviditetsvecka 32. I den nationella kohorten ”The Extremely Preterm infants in Sweden study” (EXPRESS), visades ett födelsetal på 0,2 % avseende barn födda före graviditetsvecka 27 under åren 2004 och 2007.

Dödligheten och sjukligheten är fortfarande hög bland extremt prematurfödda barn men har förbättrats stort under de senaste årtiondena. Under åren 1990 och 1992 överlevde inga barn som föddes i graviditetsvecka 22 enligt en svensk studie som publicerades 1997. Bara tio år senare visades i EXPRESS att 10 % (n=5) av barn som föddes i graviditetsvecka 22 överlevde till ett-års ålder. Under 1960/70- talen introducerades nutritionsbehandlingen först två till tre dygn efter födelsen om det prematurfödda barnet fortfarande visade livstecken. En behandlingsform som ter sig oetisk idag. För att förebygga att nutritionsbrister som kan finnas vid födelsen förvärras ytterligare, förespråkar nuvarande nutritionsriktlinjer insättning av nutritionsbehandling så snabbt som möjligt, redan inom några timmar efter födelse.

Tre sjukdomar som ofta drabbar extremt prematurfödda barn är bronkopulmonell dysplasi (BPD), prematuritetsretinopati (ROP) och öppetstående ductus arteriosus (PDA). Såväl lungsjukdomen BPD som ögonsjukdomen ROP beror på störd utveckling av lungorna, respektive ögats näthinna och är mycket vanligt förekommande hos extremt prematur födda barn. BPD kan orsaka lungsjukdom även senare i livet och den allvarligaste formen av ROP kan leda till blindhet. Sambandet mellan näringsintag och risk för BPD respektive ROP har påvisats i ett fåtal observationsstudier. Ungefär 50 till 80 % av extremt prematurfödda barn drabbas av BPD eller ROP. Ett blodkärl som kallas ductus arteriosus förbinder lungartären med stora kroppspulsådern (aorta) under fostertiden. Med hjälp av prostaglandiner hålls kärlet under fostertiden öppet eftersom cirkulation genom lungorna inte behövs. Vanligen slutar sig blodkärlet snabbt efter födelsen, redan efter första dygnet men hos prematura barn, i synnerhet de som föds inom de lägsta graviditetsveckorna är stängningen ofta fördröjd. Obehandlad PDA kan medföra förhöjd andnings- och hjärtfrekvens, matintolerans och försämrad lungfunktion. I Sverige behandlas ungefär 60 % av de extremt prematurfödda barnen för PDA.

Syftet med denna avhandling är att studera det extremt prematurfödda barnets nutritionsintag avseende energi, makronutrientier (protein, fett, kolhydrater) och vätska: i) över tid och intagets samband med tillväxt, BPD samt ROP och ii) under operationsveckan i samband med kirurgibehandling av PDA.

Samtliga studier inom denna avhandling är epidemiologiska och baserade på prospektivt insamlade bakgrundsuppgifter från EXPRESS kohorten om t ex neonatal sjuklighet, dödlighet, behandlingsinsatser och nutritions- och tillväxtuppgifter från sjukhusets patientjournaler och kvalitetsregister. Detaljerade nutritions- och tillväxtuppgifter registrerades retrospektivt i ett datorprogram för nutrition och tillväxt, Nutrium. Insamlingen och registreringen av nutritions- och tillväxtuppgifter har pågått under flera år och omfattar information om såväl parenteral som enteral nutrition, egen- och donerad bröstmjolk, enterala berikningsprodukter, samt vitamin och mineralpreparat. Mycket hög andel av egen bröstmjolk och all donator mjolk är analyserad på innehåll av makronutrientier (protein, fett, kolhydrater). Analys av bröstmjolk är en förutsättning om berikning på individuell basis ska kunna tillämpas. Dessutom inkluderades läkemedel spädda i glukos eller saltlösning, spolvätskor samt givna blodprodukter i datainsamlingen.

I studie I studerades hur nutritionsrelaterade ändringar över en åtta årsperiod (2004- 2011), påverkade nutritionsintag och tillväxt av extremt prematurfödda barn i Stockholm. Uppdaterade internationella och lokala nutritionsriktlinjer, introduktion av Start-up (en standard parenteral nutritionspåse), introduktion av Nutrium och Clinisoft (avancerat, web-baserat journalsystem), utökad anställning av dietist och utökad undervisningsfrekvens av avdelningspersonal (inte minst nutritionsassistenter), är några av ändringarna som vidtogs och implementerades under studieperiodens åtta år. Studieresultat visade förbättrat nutritionsintag och tillväxt hos barnen, i synnerhet under den senare halvan av perioden (2008-2011). Trots detta var det många barn i slutet av studieperioden som fortfarande inte mötte minimala uppskattade nutritionsbehov.

Studie II är baserad på samma kohort som studie I, men med fokus på energi- och proteinintag under den första veckan och månaden och dess påverkan på tillväxten och risken för BPD och ROP. Resultaten visade att högre energi- och proteinintag under den första levnadsveckan var förknippat med förbättrad viktutveckling. Under åren 2008 till 2011 var ökat intag av energi och protein under levnadsdag 7 till 27 förknippat med en minskad risk för BPD, vilket inte sågs för barn födda före 2008. Hos barn med färre än tio dagar i respiratorvård var ökat energiintag under levnadsdag 7 till 27 associerat med en minskad risk för ROP, vilket inte gällde för barn som hade fler än tio respiratordagar.

I studie III undersöktes nutritionen under operationsveckan som påbörjades tre dagar innan och slutade tre dagar efter kirurgisk behandling av PDA i Sverige under åren 2004 till 2007. Resultaten visade att intaget av energi och makronutrientier under den operationsveckan var lägre än uppskattat behov. Sämst var näringsintaget under operationsdagen (dag 0) samt hos barn som opererades under den första levnadsveckan. Näringsintaget förbättrades mot slutet av veckan men fortfarande på dag tre efter operation var intaget av energi, protein och fett lägre jämfört med dag tre innan operation. Det låga näringsintaget var oberoende av barnets graviditetsålder.

Studie IV är baserad på samma kohort som studie III, men med fokus på skillnader mellan de neonatala intensivvårdsavdelningarna i Sverige avseende barnens intag av energi, makronutrientier och vätska under operationsveckan. Nutritionsintaget skiljde sig mellan avdelningarna; i synnerhet avseende vätskeintag och det enterala- och parenterala nutritionsintaget, var skillnaderna stora. Avseende den parenterala nutritionens koncentration var skillnaderna mellan neonatalavdelningarna också stora men inga koncentrationsresultat nådde upp till det rekommenderade koncentrationsmålet för parenteral nutrition. Inga av inkluderade avdelningarna hade tillgång till sjukdomsspecifika nutritionsriktlinjer.

Sammanfattningsvis visar denna avhandling att det finns samband mellan nutrition, tillväxt och minskad risk för sjuklighet. Trots förbättrat nutritionsintag över tid finns det fortfarande potential för ytterligare optimering av nutritionsbehandling på Stockholms neonatala intensivvårdsavdelningar. Avsaknad av sjukdomsspecifika riktlinjer öppnar för variation mellan de svenska neonatala intensivvårdsavdelningarna, avseende nutrition under operationsveckan. Behov av konsensus beträffande nutritionsbehandling under operationsveckan föreligger. Fortsatt forskning inom neonatal nutrition behövs, inte minst med tanke på att det fortfarande är oklart hur optimal nutrition och tillväxt ska se ut för de allra minsta barnen inom sjukvården.

## 9 STATEMENT OF AUTHORSHIP

### Paper I

Vera Westin (VW) and the second co-author contributed equally to this work and drafted the initial manuscript and performed statistical analyses. VW was responsible for management and control of the nutritional data. VW had a major part in the retrospective acquisition and registration of nutritional and anthropometrical data in the nutrition calculating software for nutrition and growth (Nutrium). Detailed nutritional intakes during an eight weeks' period including 6976 individual postnatal days of 218 extremely preterm infants included in the Stockholm research study (2008-2011), were registered in Nutrium. Data acquisition and registration took several years and turned out to be very time consuming. It took up to four hours or more to register one infant in Nutrium. In 2013, the data-base of the Stockholm section of the EXPRESS cohort (2004-2007) was accomplished by VW and one of the co-authors. In total, nutritional intakes of 11136 individual postnatal days were included in the analyses, comprising the Stockholm section of the EXPRESS cohort (2004-2007) and the Stockholm research study (2008-2011). VW contributed with knowledge about nutritional components and their interaction and had also an active role in the interpretation of results. VW contributed actively in reviewing and revising the scientific content of the manuscript. VW was responsible for correspondence with the journal.

### Paper II

The cohort of paper I is also used in paper II. In collaboration with the first co-author, (VW) designed the hypothesis regarding nutrition and growth. VW was responsible for management and control of the nutritional data. VW had a major part in the retrospective acquisition and registration of nutritional and anthropometrical data in the nutrition calculating software for nutrition and growth (Nutrium). VW contributed with knowledge about nutritional components and their interaction and had an active role in the interpretation of results regarding nutritional intakes and growth. VW participated in writing parts of the method. VW contributed actively in reviewing and revising the scientific content of the manuscript and for the revision of the reviewer's comments.

### Paper III

VW drew up the design of this paper together with the last co-author and had the main responsibility regarding data of nutritional intakes. VW was responsible for management and control of the nutritional data. VW had a major part in the retrospective acquisition and registration of nutritional and anthropometrical data in the nutrition calculating software for nutrition and growth (Nutrium). During several years, 4160 individual postnatal days of 130 extremely preterm infants during an eight weeks' period, regarding the Stockholm section of the EXPRESS cohort, were registered in Nutrium. Data was later used in Paper I. The perioperative data of the Stockholm section of the EXPRESS cohort was used in this Paper. A total of 35 infants included in the Stockholm section of the EXPRESS cohort, who underwent ductal ligation for PDA were included in both Paper I and in this Paper. Since nutritional intakes were registered only once a week after the first month of life (>28 days), the registration of 35 infants' intakes, included in the sub-cohort of EXPRESS who underwent ductal ligation for PDA after 28 postnatal days, was accomplished. In total, nutritional intakes of 980 individual postnatal days of 140 extremely preterm infants were included in the analyses, comprising the sub-cohort of EXPRESS (2004-2007). VW participated in writing parts of the method. VW contributed actively in reviewing and revising the scientific content of the manuscript. VW was responsible for correspondence with the journal and for the revision of the reviewer's comments.

### Paper IV

The cohort of paper III is also used in paper IV. VW drew up the design of this paper together with co-authors and had the main responsibility regarding analysing nutritional intake. VW was responsible for management and control of the nutritional data. VW had a major part in the retrospective acquisition and registration of nutritional and anthropometrical data in the nutrition calculating software for nutrition and growth (Nutrium). VW analysed and compiled data. VW wrote the first draft of the manuscript and revised it.

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